IVF-related research

Pre-implantation genetic diagnosis

Research concerned with improving IVF

Research using human embryos

Evaluation

Gezondheidsraad
Health Council of the Netherlands

Vice-voorzitter

To the Minister of Health, Welfare and Sport
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On 24 February 1994, the State Secretary for Welfare, Health and Cultural Affairs requested scientific information about artificial reproduction techniques (letter number ZZT/TOPAZ/941055). To that end, my predecessor appointed the IVF Planning Decree Review Committee. The Committce answered the request for advice by producing three advisory reports. Having heard the Standing Committee on Medicine and the Standing Committee on Medical Ethics and Health Law, I herewith enclose 'IVF- related research', the Committee's third and final report.

This advisory report is concernced with pre-implantation genetic diagnosis (PGD) and with new techniques, which may lead to improvements in IVF. The techniques discussed are all still at the research stage. An important socio-ethical and legal question that relates to this research concerns the permissibility of the use of human embryos for research purposes. The Committee's analysis provides essential building blocks for decision-making about this.

Referring to its previous advisory reports on ICSI and standard IVF, the Committee indicates that the introduction of the techniques discussed has not always been in accordance with the reference model used for many years within the scope of MTA. A phased research path is essential here. I subscribe to the Committee's view that where medical assistance in reproduction and the improvement of that assistance is considered as socially important, the judicious development of knowledge and techniques in that field ought to be facilitated. This is the joint responsibility of government, professional organizations, and financiers.

(signed)
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Contents

Executive summary

With this report (the last in a series of three) the IVF Committee of the Health Council concludes its response to the request for an advisory report submitted in 1994. The report indicates the current level of knowledge with regard to pre-implantation genetic diagnosis (PGD) and describes current developments in the field of research concerned with improving IVF. A separate chapter is devoted to the issue of whether it is permissible to use human embryos for research aimed at further developing PGD or improving IVF. It also explores the conditions to be imposed on such research. Finally, the Committee reviews its previous reports, in terms of the extent to which the introduction of artificial reproduction technology in the Netherlands has been judiciously implemented.

Pre-implantation genetic diagnosis

Pre-implantation genetic diagnosis (abbreviated to PGD) is a method which combines genetic diagnosis and artificial reproduction techniques. In PGD, diagnosis to detect monogenic or chromosome abnormalities is carried out on the oocyte or on the embryo, *in vitro.* There are three variants: polar body biopsy (diagnosis using the oocyte), blastomere biopsy (diagnosis using the embryo, during cleavage) and trophoblast biopsy (diagnosis using the embryo at the blastocyst stage). PGD is carried out in conjunction with an IVF treatment or ICSI treatment. Depending on the result of the test, a decision can be taken to refrain from fertilization of the oocyte or from

transfer of the embryo to the womb. This technology allows the woman in question to avoid the risk of bearing a child with a given abnormality.

The polar body and blastomere biopsy forms of PGD have already been in use for about 10 years, in the context of clinical research. In the Netherlands, the University Hospital of Maastricht has been involved in this research for several years. To date, more than 100 children are born following PGD, two of them in the Netherlands.

The aim in developing PGD was to create an alternative to existing methods of prenatal diagnosis (chorionic villus sampling and amniocentesis). The scope of this technique was, initially, restricted to fertile couples with an *individually elevated* chance of a monogenic (possibly sex-linked) or chromosomal abnormality in their offspring. Currently, throughout the world, more than half of the cases in which PGD is used involve subfertile couples undergoing an IVF treatment who, as a consequence of the woman's age, have an elevated chance of conceiving a child with a chromosomal abnormality. This involves the *routine* use of PGD, also referred to as pre-implantation genetic screening (PGS).

The various forms of PGD are still in the stage of scientific research. There is still much uncertainty about various aspects of the method, particularly the reliability of the diagnosis and its safety for the offspring involved. Further research is required in this area, along with studies to determine how PGD influences an embryo's chance of implantation and the chance of an ongoing pregnancy.

The committee gives special attention to the acceptability of various forms of biopsy. It has been suggested that blastomere biopsy is equivalent to the creation of embryos for research purposes. This depends on whether, at the stage the biopsy is taken, the embryo cells can individually develop into a complete new organism. Based on the scientific literature currently available, the committee concludes that this is most likely not so.

Another ethical dilemma is the selection of embryos after PGD. Unavoidably, the question of the acceptability of taking carrier status of recessive genetic diseases as a criterion for selection comes to the forefront.

Finally, the Committee addresses the applicable legislation and regulations such as the Medical Research Involving Human Subjects Act and the Special Medical Treatments Act.

Research aimed at improving IVF

Only 15 to 20% of all initiated IVF treatment cycles lead to an ongoing pregnancy. Any increase in this chance is of enormous significance to those couples suffering from involuntary childlessness who must resort to IVF. Fewer couples will be disappointed. Women will less often have to undergo hormone stimulation and follicle

puncture, thereby reducing their exposure to the risks and distress associated with these procedures. In this connection, the Committee addresses itself to research aimed at improving the various aspects of the laboratory phase of IVF, including culture conditions.

Alleviation of the distress and risks endured by the woman can also be expected of research aimed at improvement of protocols for hormone stimulation. It would be even better if hormone stimulation could be omitted entirely. Accordingly, the Committee emphasizes the importance of further research into the possibility of culturing immature oocytes outside the woman's body until they are mature enough to be fertilized. This procedure is called *in vitro* maturation of oocytes (IVM). Research in this area has been going on for quite some time. Although children have been born after IVM, there is no immediate prospect of its introduction into IVF practice. The results obtained to date have been disappointing. In addition, there is apprehension that IVM may involve certain health risks to the offspring. The Committee believes that no further clinical research should take place until these issues have been resolved.

Similar reasoning applies to research into the possibility of keeping oocytes in frozen storage for later use (cryopreservation of oocytes). This line of research has also met with very little success. Furthermore, the technique has been introduced into clinical use before its safety has been adequately investigated in animal experiments. However, the Committee does emphasize the potential significance of this research. Should it become possible to hold oocytes in frozen storage for later use, then it will no longer be necessary to fertilize all oocytes derived by hormone stimulation. This could, to a large extent, prevent the creation of surplus embryos. The Committee considers this to be a moral gain. In addition, cryopreservation of oocytes could be important for women who are at risk of becoming prematurely infertile as a result of treatment for cancer etc. They could have their oocytes frozen to ensure that they would have the option of bearing children at a later date *(fertility insurance)*.

New methods and techniques in the field of artificial reproduction are sometimes tested in humans without adequate preclinical research in animals (for possible harmful effects, including longer term effects and effects spanning several generations). Any possible safety hazards are thus shifted to the woman and to the child. The Committee considers this to be unacceptable. Finally, the Committee points out that preclinical research using human embryos can often contribute to a further reduction of the uncertainties left by animal experiments regarding the prudence of progressing to clinical application.

Research using human embryos

In a separate chapter, the Committee gives a comprehensive description of the discussion surrounding the ethical and legal acceptability of research using human embryos. It also sets out its own position. With reference to previous advice from the Health Council, the Committee states that while a given value should be assigned to the embryo *in vitro* (by virtue of which it deserves respect), this value is relative and can be overridden when other, more imperative interests are involved. Also, the Committee does not consider the creation of embryos specifically for research to be unacceptable *a priori,* although the use of surplus embryos is to be preferred on moral grounds. The Committee considers it acceptable for embryos to be created for research purposes only when such research cannot be carried out using surplus embryos (either because of the nature of the research or because no surplus embryos are available). The requisite oocytes should be obtained in a morally sound manner.

The Committee takes the view that both the further development of PGD and the improvement of IVF treatment are of such importance to human health that, in this context, it can be acceptable to perform research on human embryos. This does not automatically mean that a concrete research proposal would be acceptable. This depends on whether the research can be expected to produce information which is relevant to these objectives and which cannot be obtained by other means. In addition, it must also satisfy the other conditions applicable to research involving human embryos. This would be judged, on a case by case basis, by the Central Committee to be set up in accordance with the provisions of the Medical Research Involving Human Subjects Act.

In certain cases, dependent on the nature of any uncertainty remaining after the completion of animal experiments and on the severity of the possible consequences, the Committee would consider unacceptable researchers refraining from preclinical embryo research or not awaiting the results of such research and proceeding directly to the clinical application of a given method or technique. Here it makes no decisive difference whether the research in question uses surplus embryos or whether it requires the specific creation of human embryos. Under no circumstances whatsoever may women and children be turned into trial subjects for the sake of protecting embryos.

Evaluation

Finally, the Committee has considered whether artificial-reproduction technology has been introduced in the Netherlands along the lines of the principle of 'evidence-based medicine'. In this evaluation, the Committee refers to analyses and conclusions from

its previous reports on ICSI and IVF. It postulates a reference model for the introduction of'special' medical technology. The Committee concludes that this introduction has not in each case proceeded entirely satisfactorily. Steps in the requisite research have been omitted. With regard to the future, the solution should involve setting priorities for research. This area is the common responsibility of government, health professionals and financiers.

Recommendations

Regarding PGD:

- For the time being, the use of PGD requires a research protocol.
  Concrete proposals for preclinical or clinical research in this field require evaluation by the Central Committee to be set up in accordance with the provisions of the Medical Research Involving Human Subjects Act.
  The Clinical Genetics Planning Decree should be declared to be applicable to the DNA research carried out within the framework of PGD.
  Clinical research should take place in centres where sufficient expertise is available in the fields of reproduction, embryology and genetics. These centres must possess a permit based on the IVF Planning Decree. Stringent requirements must be imposed on patient information and supervision.
- When formulating proposals for studies in the field of PGD, the inclusion and exclusion criteria need be no stricter than those used for prenatal diagnosis (with the exception of screening for risks related to advanced maternal age).
- If it is to be routinely offered to IVF couples, there must be more clarity about whether PGD should be regarded as population screening requiring a permit within the framework of the Population Screening Act.
  Long-term follow-up of children born through the use of PGD is required. The Committee considers it important that Dutch researchers affiliate themselves with the existing international registration of study results (as the Maastricht group has already done). This registration should be so organized that the collected data can be released for further analysis, with a view to the necessary follow-up etc.

With regard to research involving human embryos:

- In the announced law on procedures involving human gametes and embryos, it must be stipulated that proposals for research using human embryos *in vitro* require approval by the Central Committee to be set up in accordance with the provisions of the Medical Research Involving Human Subjects Act.
- Such research must, in any event, meet the following criteria:
  - it involves a major health issue;

- the research proposal is scientifically sound;
- the data required cannot be obtained in any other way;
- the research does not use any more embryos than are strictly necessary;
- no embryos are created for research which can also be carried out using surplus embryos
- the *informed consent* is obtained of those authorized to determine the fate of the embryos or (if the research implies the creation of embryos) gametes;
- the interests of oocyte-donor candidates are protected (in case of research for which embryos are created)
- embryos used for research purposes may not subsequently be transferred to the womb.

The new legislation relating to human embryos and gametes should not contain a ban on the creation of embryos for research purposes. Ratification by the Netherlands of the Treaty on Human Rights and Biomedicine of the Council of Europe should be made subject to a proviso on this point.

Chapter 1

Introduction

1.1 The request for an advisory report

On 28 February 1994, with a view to updating the IVF Planning Decree and the relevant future legislation, the then State Secretary for Welfare, Health and Cultural Affairs requested the Health Council of the Netherlands to prepare a scientific report on *in vitro* fertilization. The request for an advisory report (annex A) is broad in its scope. Besides being asked for its opinion on current IVF practice, the Committee was also requested to provide advice on new developments, including the use of intracytoplasmic sperm injection (ICSI) for diminished fertility of the man and IVF in combination with pre-implantation genetic diagnosis (PGD). The Committee was also asked to indicate which new ethical and social issues are related to these developments.

1.2 The Committee

On 10 May 1994, the then vice-president of the Health Council installed the IVF Planning Decree Review Committee (hereinafter referred to as the Committee) and instructed it to draw up recommendations in answer to the State Secretary's request. The Committee met 29 times (May 1994 - February 1998). Dr MHEC Pieters, an expert in embryology and genetic diagnosis, joined the Committee in connection with the subjects raised in this third advisory report. The Committee was advised by two external experts. Details of the Committee's composition are provided in annex B.

1.3 Structure of this advisory report

This advisory report is the third and final one in a series of three which address the issues raised in the request to the Committee for an advisory report. The first was published on 19 June 1996 and was entirely devoted to ICSI (GR96). The second, published on 10 February 1997, contained the building blocks for a new IVF Planning Decree (GR97a). In this third advisory report, the Committee discusses various new aspects of IVF research.

Firstly, there is an extensive discussion of the current level of knowledge with regard to the development of pre-implantation genetic diagnosis (PGD). This is concerned with a method that is not in itself part of the modern arsenal of artificial-reproduction techniques but which is being developed in close relation with IVF and is inconceivable without the latter technique. The Committee then provides a brief and by no means complete summary of research into improving existing IVF treatment. The improvements should lead to a higher rate of success, less distress and lower risks for the woman. The question of whether it would be responsible to introduce new methods and techniques into clinical practice can only be answered on the basis of preclinical research. The Committee points out that research into artificial reproduction can be conducted on animals as well as on human embryos. This raises the question of whether it is acceptable to use human embryos for scientific research and, if acceptable, under what conditions. The Committee discusses this in a separate chapter.

The Committee has specifically limited itself in this advisory report to a discussion of research concerned with the further development of PGD and with improving IVF. A similar approach was also taken in the chapter on the acceptability of embryo research. This means the Committee has refrained from expressing opinions on numerous subjects that may well be connected with human reproduction, but which are beyond the scope adopted for the present advisory report. This also applies to a subject that is currently attracting a great deal of interest, namely cloning. The advisory report only refers indirectly to cloning, namely when discussing the question of whether the likelihood of successful IVF treatment can be increased for some patients by means of embryo splitting (3.1.5).

The Committee concludes its advisory role with a consideration of the degree to which the introduction of artificial-reproduction technology has proceeded judiciously in the Netherlands. In this evaluation, the Committee refers to the analyses and conclusions in its two previous advisory reports.

A definition of terms is provided (annex C), as the various methods and techniques cannot be described without using technical language.

Chapter 2

Pre-implantation genetic diagnosis

2.1 Introduction

Pre-implantation genetic diagnosis (PGD)* is a method intended to detect monogenic or chromosomal aberrations in the oocyte or in the embryo *in vitro.* PGD therefore necessitates IVF treatment**. If the result of the test gives cause, either transfer of the embryo to the womb or fertilization of the oocyte does not go ahead. This method allows the woman to avoid the risk of bearing a child with the abnormality concerned. In 1989, The Health Council reported the first use of PGD in an English clinic (GR89).

This chapter contains an extensive discussion of the current level of knowledge. It examines the reliability of PGD, the method's safety for offspring and the question of the possible target groups for which PGD is being developed. Specific ethical questions concern the method's acceptability (the removal of cells from human embryos) and the selection criteria for transferring embryos after PGD.

The Committee realizes that the development of PGD also raises the wider social question regarding the implications of genetic testing intended to prevent genetic abnormalities in offspring. However, it does not believe its task is to examine this in

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* The method is also known as pre-implantation diagnostics (PID). The Committee uses the more comprehensive name in accordance with the international literature. The abbreviation PID led to confusion with the corresponding abbreviation used in gynaecology for Pelvic Inflammatory Disease.

** The literature also refers to the uterine lavage technique as an alternative to using embryos produced using IVF. Flushing embryos from the womb that have been produced by natural fertilization provides embryos at the blastocyst stage for genetic diagnostics (Bus85, Mug93). Major risks are associated with uterine lavage (GR92).

the present advisory report. The Committee assumes that a wide consensus exists in the Netherlands about the acceptability of genetic testing, which is intended to provide individuals with information they can use as a basis for making well-considered decisions about risks to their own health and that of their offspring (GR94a). This does not of course settle the discussion of the advisability of further increases in the possibilities for any such testing. There is a reference to the paradox that the availability of prenatal testing can result in a reduction rather than an increase in the freedom of choice of couples with a greater risk of having a disabled child (Rei96). More specifically in relation to PGD, there is a warning of the danger of a slippery slope towards eugenic selection of embryos (CCN86, Pau97, Tes92). The Committee believes these issues should be part of the wider social discussion of the introduction of new types of genetic testing.

This advisory report also leaves aside the legal issues generally associated with genetic testing, such as the issue of protecting privacy with regard to genetic information and handling genetic data within the scope of examinations and insurance. Various Health Council Committees have addressed these issues (GR89, GR93, GR94a).

2.2 Three forms of PGD

The aim of developing PGD was to create an alternative to the methods of genetic diagnosis that have been common for many years in prenatal health care, such as chorionic villus sampling and amniocentesis. An important advantage PGD is considered to have over prenatal diagnosis is that the couple is not faced with deciding whether or not to terminate a pregnancy in the event of an unfavourable result. After all, in PGD the diagnosis can take place before pregnancy. The Committee returns to the relationship between PGD and prenatal diagnosis later in this chapter.

When the Health Council's Genetics Committee conducted an exploratory examination of PGD in 1989, its comments were mainly concerned with diagnoses in embryos at the four- to eight-cell stage of development (GR89). This is only one form of PGD. The literature now refers to three forms of PGD. These are different with regard to the moment at which the cell material is taken for genetic testing. The technical terms used below are explained in annex C.

1 In *polar body biopsy,* the diagnosis is made before fertilization, which is why the method is also known as preconception genetic diagnosis. Various oocytes are obtained after hormone stimulation. The first polar body is removed from these as yet unfertilized oocytes in order to carry out the diagnosis. The oocytes that are 'free' of the abnormality being sought qualify for fertilization. The diagnosis can be repeated for the *second* polar body. However, this only appears after

fertilization. In that case, the procedure can no longer be referred to as preconception diagnosis. (References below to polar body biopsy mean the biopsy of *the first* polar body.)

2 In *blastomere biopsy,* genetic diagnosis takes place on one or two cells taken from the embryo when it consists of six to ten cells (blastomeres), which is usually on the third day after fertilization. Only those embryos that are free of the abnormality being sought qualify for transfer to the womb.

3 In *trophoblast biopsy,* the cells for the diagnosis are taken at the blastocyst stage (human embryos reach this stage approximately five days after fertilization). This method is also known as 'late biopsy', as distinct from 'early' blastomere biopsy. A distinction can be made in the blastocyst stage between the cells from which 'the embryo proper' will develop, the embryoblast, and cells that will play a role in the contact between the embryo and the outside world, the trophoblast cells. In late biopsy, cells are only removed from the trophoblast, hence the name trophoblast biopsy. The cells from which the embryo proper will develop are untouched. Another advantage would be that a reasonable number of cells can be obtained (e.g. ten), which simplifies the diagnosis.

2.3 Fields of application

Preclinical and clinical research has been underway for approximately 10 years into the polar body biopsy and blastomere biopsy variants of PGD. The research currently involves fourteen centres around the world (Har96b, Ver93). PGD is mainly used in the United Kingdom, the United States and Belgium, but has also been used in the Netherlands in recent years. More than one hundred children have now been born after PGD (Han97).

The clinical research originally focused on couples with an individually greater likelihood of having a child with a severe monogenic or chromosomal abnormality. PGD could provide them with a new option in addition to or instead of prenatal diagnosis.

Because in the case of polar body biopsy, the diagnosis is carried out on genetic material derived solely from the woman, it is only worthwhile for detecting abnormalities that are (also) transmitted via the mother. This form of PGD is used for carrier couples of cystic fibrosis, alfa-1-antitrypsin deficiency, Tay-Sachs disease, retinitis pigmentosa and for carriers of haemophilia A. A single research group in the United States is responsible for the leading publications on polar body biopsy (Ver90, Ver94).

Research into blastomere biopsy initially focused on preventing certain *sex-linked* hereditary abnormalities by selecting embryos on the basis of sex. In 1989 a research

group at London's Hammersmith Hospital announced that it was the first to employ the method successfully (Han89, Han90). The group is now the most experienced in blastomere biopsy (Han96). Other research groups outside the Netherlands have also concentrated on blastomere biopsy, as has the only research group in the Netherlands in this field (see 2.4). The diagnostic process has expanded to include *non-sex-linked* monogenic abnormalities. Cystic fibrosis accounts for most of the work (Ao96, Han92). Other abnormalities for which tests have been carried out within the scope of blastomere biopsy include Tay-Sachs disease, Lesch-Nyhan syndrome, haemophilia, Duchenne muscular dystrophy, fragile-X syndrome, alfa-1-antitrypsin deficiency, retinitis pigmentosa, thalassaemia, familial polyposis coli, myotonic dystrophy, and Huntington's chorea (Gri92, Han97, IWG94, IWG96, Lis97, Sch96a).

A further PGD research field was added around 1992. Research using surplus embryos revealed that many embryos produced after IVF displayed chromosomal abnormalities. The occurrence of aneuploidy in particular was striking in embryos in the cleavage stage (Del93, Mun93). This was largely accounted for by a direct link between aneuploidy in the embryo and the age of the woman (Mun95a, Mun95b, Mun95d). The high frequency of chromosomal aberrations in embryos in an early stage of development gave rise to the idea that *routine diagnosis* of abnormalities in couples who undergo IVF treatment for a fertility problem would be worthwhile (IWG94). Research in this field has mainly focused on couples with an increased risk of certain chromosomal aberrations in the embryo, owing to the age of the woman (IWG96, Man96, Ver94). It is hoped that this research will help bring about improvements in the likelihood of IVF being successful in the group concerned. There are two lines of research underway; one focuses on polar body biopsy and one on blastomere biopsy. The first is concerned with detecting certain chromosomal aberrations (aneuploidy) in oocytes, i.e. before fertilization (Mun95c, Ver96b). The second is concerned with detecting chromosomal aberrations that arise after fertilization (Gia97). It is sometimes suggested that the two methods of biopsy should be combined (Reu96, Ver96a). Clinical PGD research has mainly concentrated on this field of application (IWG96).

Research into trophoblast biopsy is as yet still limited to preclinical research, i.e. *in vitro* culture of surplus human embryos to the blastocyst stage (day 5 to 6 after fertilization), with the aim of being able to carry out the biopsy. It can be concluded from the limited available data that extended culture is successful for relatively few embryos. The result is that only a small number of embryos are available for diagnosis and an even smaller number for transfer (Dok90, Geb95, Pic95, Ver94).

2.4 Situation in the Netherlands

In the Netherlands, the only centre involved in developing PGD is the University Hospital of Maastricht. The research plans received publicity in 1988. The discussion of the plans was a major reason for the establishment in 1989 of KEMO, the Provisional Central Ethical Review Board, by the then Minister of Public Health (EK88). In 1990, a proposal for research into blastomere biopsy was submitted to KEMO by the Maastricht researchers. The proposal was limited to animal-based experimental research and research using human gametes and surplus embryos. KEMO agreed to the research protocol, subject to certain conditions (KEM91). In 1994, positive advice was given by KEMO on the clinical part of the research (KEM94). During the same period, the Maastricht research plans became the subject of a political debate, also in connection with the preparation of a bill on the use of human gametes and embryos (Official Report, Lower House 93-94, 23016). The Maastricht University Hospital began research into blastomere biopsy in clinical practice in 1995. They adopted a protocol with stringent inclusion criteria, in line with the aforementioned KEMO advisory report: severe sex-linked conditions, cystic flbrosis and fragile-X syndrome (Eve97). In Maastricht, blastomere biopsy within the scope of research has currently been used for 22 couples, mainly for the diagnosis of sex-linked hereditary conditions. It involved 33 cycles in which follicle puncture, biopsy and diagnosis were possible, and 27 transfers of healthy embryos, which resulted in 5 ongoing pregnancies and 2 births (verbal information from dr JPM Geraedts).

As far as the Committee is aware, several other university centres in the Netherlands are interested in the development of PGD, but they have not yet conducted any research.

2.5 Effectiveness

Because PGD unites genetic diagnosis and artificial reproduction, the Committee distinguishes between two aspects of effectiveness, namely, the level of reliability of the diagnosis and the likelihood of a pregnancy leading to the birth of a healthy child.

2.5.1 Reliability of the diagnosis

PGD's reliability largely depends on the reliability of the genetic diagnosis method used. If the diagnosis is false-positive, the embryo is mistakenly not transferred. A false negative diagnosis signifies a failure to achieve the intended risk reduction.

In PGD, genetic diagnosis is complex because it has to be performed on a very small amount of cell material, usually taken from just one cell, and because little time is available (1 to 2 days) for transferring the embryo that is kept in culture. PGD therefore only became possible once certain DNA techniques had been developed. One such technique was PCR (polymerase chain reaction). PCR enables a cell's genetic information to be multiplied (amplified), hence the alternative name DNA amplification. This method of DNA diagnosis has been used in PGD right from the start.

Another diagnostic technique, used in PGD since 1992, is known as FISH: fluorescent in-situ hybridisation*. This technique is based on recognizing larger specific DNA fragments in the cell, which can be seen under the microscope. Thanks to considerable improvements, this technique has made a major contribution to PGD development. FISH enables the diagnosis to be made in one working day.

Each of the diagnostic techniques has its own field of application. PCR is mainly used for detecting monogenic conditions (gene mutations). FISH is used for detecting (numerical and structural) chromosomal aberrations and for sex determination.

PCR and FISH are not 100 percent reliable. Incorrect diagnoses may occur in PCR as a result of a failure in the amplification process. The literature mainly draws attention to the possibility of the amplification process only involving one of the two alleles (Gri96, Han96, Lis97, Str94). Another possible cause of an incorrect diagnosis (false-positive or false-negative) is contamination, by a sperm cell for example. Neither of these potential problems with PCR occurs in FISH. FISH may result in false-negative diagnoses owing to the problem of overprojection, amongst other things. This applies if the fluorescence signals are so close together that it is difficult to make an accurate assessment.

Much has been done over the years to increase the reliability of PCR. In blastomere biopsies for example, researchers started to carry out diagnoses on two blastomere-stage embryo cells, if possible (Gri96, Han96). Sperm contamination is avoided by using ICSI as the method of fertilization (IWG94).**

Besides the technical shortcomings, biological factors can also lead to incorrect diagnoses. In this regard, the literature points out the problem of mosaicism (Har95, Mun94). This phenomenon means that the embryo cells have a different chromosomal make-up. It may be concluded that in many cases of blastomere biopsy (possibly 25 to 50%), the cells used for the diagnosis are not representative of the entire embryo

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* The Committee only indicates the most familiar and most widely used methods of diagnosis here: PCR and FISH. Other variations of these such as multiplex PCR, PEP (primer extension preamplification) and PRINS (primed in-situ labelling) are not considered.

** The Committee understands from verbal sources that ICSI is the fertilization method now chosen by most centres in those cases involving PCR diagnosis.

(Har95, Har96a, Mun94). Further research in this field is essential. It will be necessary to obtain clarity about the implications of mosaicism for the blastomere variant (and also the trophoblast variant) of PGD. Examining two cells does in any case seem necessary, if possible. This would entail only using embryos that are at least at the eight-cell stage. An essential question is to what extent mosaicism impedes normal embryonic development. Mosaicism would be a minor problem for blastomere biopsy, if the deviant cells were not involved in the development of the inner cell mass, from which the embryo proper develops (Jam94, Mun94).

The biological problem of'crossing over' plays a role in polar body biopsy, if it is concerned with diagnosing monogenic conditions (Ger94). Ordinarily, the first polar body and the oocyte can be considered as each other's genetic counterpole. This means that if the gene mutation (the mutant allele) is found in the polar body, it is not in the oocyte. In that case, the oocyte qualifies for fertilization and subsequent transfer. However, an exchange of genetic information of the alleles may have taken place in a chromosomal pair during the oocyte's maturation divisions. This is known as crossing over. New combinations of genes occur (recombination). This can result in the first polar body and the oocyte not being each other's genetic counterpole. Diagnosis based on polar body biopsy is then inadequate (AFS94, Coh91).

In addition to the diagnosis based on the first polar body, some centres outside the Netherlands also carry out a diagnosis based on the second polar body (IWG96, Ver95a), which is only produced after fertilization. The literature has not yet established whether a diagnosis based on the second polar body in addition to the first polar body constitutes an adequate procedure, and the extent to which any such procedure would be preferable to blastomere biopsy for certain abnormalities (Ang94).

2.5.2 *Likelihood of an ongoing pregnancy*

The literature shows that more than one hundred children have been born worldwide following the successful employment of polar body or blastomere biopsy (Han97). The literature provides very little insight into each embryo's chance of implantation and the likelihood of an ongoing pregnancy after PGD. The possibility of PGD having a negative effect on this in comparison with normal IVF cannot be excluded (Har96b). Further research is necessary to determine PGD's position vis-a-vis prenatal diagnosis and to obtain an insight into the issue of how defensible the *routine* use of PGD is in (older) IVF patients (Reu96). Any such research would only be worthwhile if proper records of the data were kept, and factors such as the woman's age and the number of embryos being transferred were taken into account.

2.6 Possible harmful consequences

2.6.*1* *For the embryo*

PGD's development was preceded by animal-based experimental research and research using surplus human embryos. However, this was mainly limited to *blastomere biopsy*. Research was conducted to determine whether removing one or more cells from blastomere-stage embryos involved any hazards for the embryo *in vitro*. Research using mouse embryos revealed that biopsy at the eight-cell stage involves no hazardous consequences for the embryo's further development (Tak92). Research using surplus human embryos indicated that biopsy results in cleavage retardation (Fir91, Tar92). The embryo remaining after biopsy is generally capable of developing into a mature foetus after implantation (Pie97).

Much less is known about the possible hazardous consequences of removing the polar body within the scope of *a polar body biopsy*. In the literature, animal-based experimental research and research using surplus human embryos lead to the conclusion that removing the polar body does not appear to have a detrimental effect on the likelihood of oocyte fertilization. There is also no reported harm to the cell division process after fertilization (blastocyst formation) (Ver91, Ver94). The Committee believes further preclinical research is required before allowing this form of PGD to be introduced as part of clinical research.

In theory, it seems plausible that *trophoblast biopsy* would have no harmful effect on the embryo because the cells that form the embryo remain untouched in this form of PGD. This has provisionally been confirmed by the scarce research results available in this field, especially from animal-based experiments (Per91). It goes without saying that further preclinical research is necessary before this form of PGD can be introduced into clinical practice.

2.6.2 *For the child*

As mentioned, PGD is used in combination with IVF, or in certain cases with ICSI. The possibility cannot be excluded of artificial fertilization techniques affecting the resultant children. The Committee has addressed this issue in detail in previous advisory reports, and called for follow-up research (GR96, GR97a). The degree to which PGD adds to the possible harmful effects is unknown. The number of children born after the use of blastomere biopsy and polar body biopsy is too small to enable definite conclusions to be drawn. Moreover, these children are still very young. Literature on this point is very meagre (Gri94, Sou96a, Sou96b). The Committee

believes further research into the possible harmful consequences for the children is essential. Proper research in this field implies paying attention to the right control groups. It is necessary for the centres using PGD around the world to collect their data in accordance with uniform guidelines on the classification of congenital abnormalities, the definition of pregnancy duration, long-term effects, etc. The data should be incorporated in the existing PGD records of the international working group on PGD.

2.6.3 *For the woman*

The possible harmful effects and risks PGD involves for the woman are entirely attributable to the IVF treatment required. See the Committee's previous advisory report on this point (GR97a). There is no question that PGD is a distressful method for the woman. The Committee believes that the psychosocial distress in particular needs to be examined. A distinction should be made between women who are fertile and undergo PGD as a possible alternative to prenatal diagnosis, and women who have a medical indication for IVF (or ICSI) and undergo PGD on the grounds of their age. The Committee believes that the literature on PGD research data should pay more attention to the distress women experience from PGD (Mie93).

2.7 Possible future target groups

The Committee indicates in section 2.3 that the use of PGD has widened in scale over the years. Research was originally conducted in the field that is comparable with the indications at the *individual* level for current forms of prenatal diagnosis. It has widened in scale to include the *routine* use of PGD for couples with a fertility problem, with a view to taking into account chromosomal aberrations related to the woman's age. The Committee therefore distinguishes between three possible target groups for PGD:

1. Normal fertile couples with a demonstrable individually increased likelihood of having a child with a monogenic or chromosomal aberration and who prefer PGD to prenatal testing. There may be various reasons for such a preference. A couple may object in principle to induced abortion. By choosing PGD rather than prenatal testing, the couple would avoid being faced with the decision of whether or not to have an abortion carried out. Moreover, a couple may have faced the choice of induced abortion previously and possibly more than once, after the results of prenatal testing. These situations create a lot of psychological distress.

The Committee believes the further development of PGD for this target group is justifiable. In such cases the aim of PGD would be to prevent severe suffering of children and their parents. In the Committee's view, the parents should be the ones to choose between prenatal diagnosis and PGD. The choice involves complex factors such as the aforementioned fact that prenatal diagnosis can lead to being confronted with the abortion issue on the one hand and, on the other, the fact that PGD also involves 1VF and is still at the research stage. The latter fact also means that, for the time being, using PGD does not remove the grounds for prenatal testing. As long as PGD is at the research stage, prenatal testing should be offered to the couple along with sufficient information about its importance vis-a-vis the risks. The Committee is not yet able to see whether PGD could in future actually replace all or part of prenatal testing (Bal97), and believes PGD may well become an additional option besides existing types of prenatal diagnosis. Even if the diagnosis is not entirely reliable, and therefore requires confirmation in prenatal testing, the use of PGD can be expected to result in parents less often being faced with the choice of whether or not to terminate the pregnancy.

The Committee has considered whether there is any reason to interpret the present field of application differently in the case of PGD than for the current practice of prenatal diagnosis. It has examined the KEMO recommendations on the PGD research conducted in Maastricht. Owing to the lack of clinical research experience available at the time and all the uncertainties that were associated with PGD, KEMO recommended limiting clinical research for the time being to severe, untreatable hereditary conditions (KEM91, KEM94). The Committee believes on the basis of the present level of knowledge there is no reason for maintaining KEMO's recommended limits on current clinical PGD research. The Committee's view is that, in principle, as far as the inclusion criteria are concerned, PGD research can now be equated with the current medical indications for prenatal diagnosis, with the exception of those related to the woman's age (Wer97)*.

The Committee stresses that PGD should still, for the time being, be conducted within the framework of a research protocol. Specific research proposals should be assessed on scientific and ethical grounds. In view of the current level of knowledge and the severe lack of expertise in the field of PGD in the Netherlands, the Committee believes this assessment should be carried out by the Central Committee to be set up in accordance with the provisions of the Medical Research Involving Human Subjects Act.

* The current indications for prenatal diagnosis are laid down in an agreement concluded in November 1995 between Dutch health care insurers and the Clinical Genetics Association.

2. Couples who qualify for IVF or ICSI treatment because of a fertility problem and who also have a demonstrably increased risk of having a child with a genetic abnormality. As with the previous target group, the aim of PGD here is to prevent severe suffering of children and their parents. The Committee therefore also believes the further development of PGD for these target groups is justifiable. It envisages a field of application that corresponds with that for the above group of patients. Here too, specific research proposals are required that should be assessed on scientific and medico-ethical grounds.

3. Couples who qualify for IVF or ICSI treatment because of a fertility problem, when PGD is used *routinely* because of possible chromosomal abnormalities in the embryo as a result of the woman's age. This form of PGD is also known as pre-implantation genetic screening (PGS) (Wer95). PGS can have two objectives. In the first place, it enables the couple to be informed about the presence of any chromosomal abnormalities in the oocytes available for fertilization (polar body biopsy) or the embryos available for transfer (blastomere biopsy). This makes it possible to prevent the woman from becoming pregnant with a child with a given chromosomal abnormality. The second objective is to increase IVF's likelihood of success by employing an extra means of embryo selection. It is after all common for pregnancies resulting from IVF embryos to end in miscarriage at an early stage (first trimester). Avoiding the transfer of chromosomally abnormal embryos would mean that even in older women it would not be necessary to transfer more than two embryos, rather than three in accordance with the current transfer policy (GR97a). This would also enable a further reduction in the number of multiple pregnancies that result from IVF.

With regard to the first objective, the Committee believes the parallel with current, generally accepted, prenatal screening in connection with the age of the woman is largely applicable. With regard to the second objective, the Committee endorses the importance of improving the likelihood of IVF being successful and reducing the number of multiple pregnancies. However, the Committee stresses that worldwide research into the value of PGD in this context is only at an early stage (2.3). Although the possibility cannot be excluded of PGS being important also for younger women undergoing IVF treatment, current research focuses on the group of women with an obviously increased risk of aneuploidy in the embryo. The Committee assumes that, as with prenatal diagnosis, any specific research plans in this field of application will be limited to women aged 36 or older for the time being. In connection with this, research into the implications of mosaicism for the reliability of the diagnosis of chromosomal abnormalities is essential.

2.8 Acceptability of invasive procedures in the various forms of biopsy

Taking cell material for diagnostic testing is an inherent part of PGD. Biopsy implies subjecting the oocyte or embryo to an invasive procedure. The Committee discussed the possible harmful consequences of this in section 2.6. Here, the Committee discusses the question of whether the invasive procedure as such is acceptable. *Polar body biopsy* uses the first polar body, a sister cell of the unfertilized oocyte. Neither the oocyte as such nor the polar body have a claim to any special protection. Because the polar body plays no role in fertilization and is lost anyway, the Committee sees no moral objections to this type of biopsy. If diagnostics are carried out on the second polar body, fertilization has already occurred. Assuming that the biopsy has no consequences for the fertilized oocyte (this remains intact), the Committee considers this type of biopsy to be comparable with the biopsy on the first polar body from the moral point of view. Nor does the invasive procedure required within the context of *trophoblast biopsy* give the Committee cause for any moral objections. In this type of biopsy, cells are taken from the part of the blastocyst (the trophoblast) from which development-assisting tissues such as the placenta arise. The cells that directly contribute to forming the embryo proper are left unharmed. The Committee believes this type of PGD may be considered as chorionic villus sampling carried out at a very early stage.

The invasive procedure required for *blastomere biopsy* engenders more discussion. It has been claimed that embryo cells at the stage used for this type of biopsy (the cleavage stage) are already 'totipotent' (CDA92). If this is interpreted as meaning that each of those cells could develop into a separate organism, each of the cells removed from the embryo would also have to be considered as an embryo (CDA92, Har94). This would mean the blastomere biopsy type of PGD not only involved removing a number of cells from the embryo but that the removal of each cell gave rise to a new embryo, which would then be subjected to an invasive diagnosis that it could not survive.

The term 'totipotency' is used with various meanings, which are not properly distinguished in discussions about the acceptability of PGD (Mau96, McL97)*. In developmental biology the term is generally understood to mean the ability of a cell to contribute to the formation of all the different somatic tissues, including the germ line. The term 'pluripotency' is also used for this. Totipotency or pluripotency is a characteristic of all the separate cells (blastomeres) of the early embryo at all stages of cell division (2-cell, 4-cell, 8-cell, etc.). The cells of the inner cell mass of the

* This discussion is only concerned with totipotency as a characteristic of the cell as a whole (cellular totipotency), not totipotency as a characteristic of the cell nucleus (nuclear totipotency) (Edw97).

blastocyst (embryonic stern cells) are still totipotent in this sense (GR97b, Mau96). However, when the term is used in the former sense mentioned, i.e., meaning that each of the cells can develop separately into a completely new organism, it is concerned with a characteristic that is lost at a much earlier moment in the embryo's development. When it still is and is no longer possible to speak of totipotency in this latter sense differs from species to species and depends on the number of cells that make up the embryo at the moment of blastocyst formation (McL97). In the case of mice, totipotency in this sense ceases to apply after the 2-cell stage, whereas in the case of rabbits and sheep, the term can still be used for embryos of 5 to 8 cells. The correspondence between mice and humans in terms of the pattern of early embryonic development (relatively early blastocyst formation) makes it plausible that the separate cells (blastomeres) of human embryos also lose their totipotency (in the latter sense) after a few cell divisions. A further indication of this is provided by results of research using human embryos; with a view to obtaining more cell material for PGD, cells that had been biopsied at the 8-cell stage were proliferated as far as possible. Although some of these cells divided several more times, no normal embryonic development occurred in any of the cases (Geb95).

The Committee concludes from the above that, in all probability, totipotency (interpreted as the ability of cells to grow separately into a new organism) no longer applies in humans at the stage (6 to 10 cells) in which blastomere biopsy usually takes place. However, on the basis of the available data, it is impossible to say with certainty that cells taken from the embryo at that stage do not have this capacity. This means that in the moral evaluation of blastomere biopsy PGD, it is necessary to take into account the unlikely possibility that carrying out the biopsy is equivalent to producing a new embryo, exclusively for use in diagnostic testing.

However, the Committee does not believe this implies that this form of PGD has to be considered as morally unacceptable. In the Committee's view, if carrying out the biopsy implies producing a new embryo, the implication has to be weighed against the moral importance of carrying out the diagnosis (Wer95). See chapter four for a more detailed analysis of this argument, which deals extensively with the issue of whether it is morally acceptable to produce embryos purely as research material.

2.9 The selection of embryos

PGD implies the selection of embryos for transfer. From the point of view of PGD, i.e. preventing the birth of a child with a particular genetic abnormality, embryos are selected on the basis of the absence of a particular gene mutation or particular chromosomal abnormalities, or the basis of sex, in order to prevent a sex-linked condition in the child. These grounds are acceptable in the Committee's opinion

because they are concerned with preventing suffering by the future child and the parents.

Because PGD is used in conjunction with IVF, the embryos are also examined for external morphological characteristics before transfer. This is justified in terms of the quality of IVF treatment.

In the Committee's opinion, there are two grounds for selecting embryos in connection with PGD that obviously require greater consideration. Is it acceptable to decide not to transfer embryos that are only carriers of a recessive hereditary condition (i.e. the future individual does not have the condition but may pass on the genetic defect to the next generation)? Is it justified when parents wish to use PGD to determine the sex of the future child, if there is no medical reason for doing so?

The Committee points out that the issue of the acceptability of taking carrier status of recessive genetic diseases as a criterion for selection is not entirely new. It also came up in connection with prenatal testing. However, it has a different connotation in PGD: selecting the embryos for transfer is a direct responsibility of the doctor and the information on carrier status is usually part of the diagnostic test report. In prenatal diagnosis, the doctor is essentially in an executive position; he leaves the choice to the parents and usually accepts their decision. It is conceivable that the parents would consider the induced abortion of a healthy foetus unacceptable, if it were only for its status as a carrier of a given condition.

The Committee is not opposed in principle to taking carrier status of recessive hereditary conditions as a criterion in PGD, as it can prevent future suffering. What the Committee does see as a problem is situations in which the most suitable embryos for transfer are carrier status embryos and selection based on carrier status leaves only qualitatively less good embryos available for transfer. Considering their responsibility for ensuring quality, doctors may object to transferring the latter embryos. On the other hand, parents could insist on starting a new stimulation cycle. The Committee believes it is relevant to make a distinction on this point between the carrier status of various types of recessive hereditary conditions (Wer95):

- carrier status of an autosomal recessive hereditary condition (e.g. cystic fibrosis)
- carrier status of a sex-linked recessive hereditary condition (e.g. Duchenne's disease).

The likelihood of the children of carriers in the first group developing the disease is small (less than 1%), because there is only a realistic likelihood if the partner is also a carrier of a mutation on the same gene. The second case involves a considerable risk of female carriers passing on the disease to their children, namely 50% for each son.

The Committee believes that no new stimulation cycle ought to be started in principle, if morphologically good embryos are available that are carriers of an

autosomal recessive hereditary condition. This is based on the consideration that the likelihood of causing suffering to the offspring is far outweighed by the risks and distress associated with a new treatment cycle. On the other hand, where carrier status in the case of a sex-linked recessive hereditary condition is involved, the risk associated with a new cycle is outweighed by the likelihood of creating suffering for the offspring as a result of transferring carrier status embryos. In that case, the Committee would be in agreement with a decision to start a new cycle.

A few years ago, the Health Council published an advisory report on sex selection for non-medical reasons (GR95). The report examined the possibility of the doctor involved in the selection of embryos for transfer after PGD being requested by the parents to take into account their preference for a boy or a girl (sex-selective transfer for non-medical reasons). The Committee concurs with the position taken in the previous advisory report. A distinction has to be made between the situation in which the sex is known and, without any further procedure being required, can be incorporated into the selection process, which precedes transfer anyway, and the situation in which an additional sex diagnosis would be required. The first case only involves asking the doctor to respect the preference of the parents, something which the Committee believes the doctor should be allowed to do so. The situation is different when determining the sex requires a separate procedure for which there are no medical grounds.

The Committee points out that the couple should be properly informed beforehand about the centre's adopted criteria for the selection of embryos for transfer after PGD. Details must also be provided of what the procedure will be in the event of there being no informative result from the diagnosis.

2.10 Legislation and regulations

The Committee calls for attention to be paid to the legislation and regulations that apply to the development of PGD.

In the first place, it is relevant that the three types of PGD discussed are considered as still being at the medical research stage. If a given type of PGD is offered within the scope of clinical research, it will be subject to the forthcoming Medical Research Involving Human Subjects Act (EK98). The concrete research proposal would then have to be evaluated pursuant to the criteria of that Act. The Act contains provisions for a Central Committee to evaluate medical research about which there is a scarcity of expertise (section 2, subsection 2b). The Committee believes this applies to PGD: for the time being, research in this field ought to be evaluated by the Central Committee. As yet, a proposal for research using human embryos (preclinical research) is beyond the scope of the Medical Research Involving Human Subjects Act;

any such research would be subject to the announced legislation on the use of human gametes and embryo's. The Committee considers it necessary for the Central Committee to evaluate any concrete proposals for such research.

Regulations of another type that apply to PGD are ministerial regulations based on section 2 or 5 of the Special Medical Treatments Act. These provisions replaced section 18 of the Hospitals Act (Stb97). Clinical research into PGD is currently only allowed in centres that are licensed under the IVF Planning Decree. The current decree is expected to be revised soon.

The Committee points out that DNA testing carried out in connection with PGD does not currently fall within the scope of the Clinical Genetics Planning Decree (of 30 March 1994). The Committee believes that a broadening of that scope would be in keeping with the objectives of the planning decree. Should PGD ever progress beyond the research stage, a licensing system based on section 2 of the Special Medical Treatments Act would be advisable, in view of the importance of grouping technical and counselling expertise and in view of the importance of protocols in connection with the ethical and social aspects.

Finally, the Committee has considered the question of whether the Population Screening Act is applicable to PGD (Stb92). The fact that PGD still has to be offered within the framework of research for the time being does not mean that this question is irrelevant. The Act also applies to population screening that is likewise scientific research. The Population Screening Act contains provisions for a licensing system for hazardous population screening. The Act's general scope is broad: population screening is considered to be involved if a medical examination is conducted in order to fulfil an offer made to a group of persons with the intention of investigating diseases or risk indicators for the benefit of the examinees. This covers genetic screening, as well as the prenatal screening of women aged 36 or older. In this context, the Committee does not exclude the possibility of PGD being covered by the Population Screening Act, if it is provided *routinely* within the framework of IVF (PGS). The Committee believes the parallel with genetic screening and prenatal screening would apply if PGD were to be used for women who, because of their age, are more likely to have children with given chromosomal abnormalities. However, it is unclear whether the Population Screening Act actually applies. The definition of population screening is currently limited to the testing of persons. If PGD were considered as the testing of embryos (rather than the woman), the technique would not be covered by the Act. The second question would be whether this type of population screening ought to be licensed, because the Act requires licensing only for screening for conditions that are not preventable or treatable. It could be argued that prevention is possible with PGD because affected embryos are not transferred. The Committee believes clarity should be provided as to whether or not the Population Screening Act applies, and

recommends that this question be included in the announced evaluation of the Population Screening Act (TK97).

Chapter 3

Research concerned with improving IVF

In this chapter, the Committee discusses various developments concerning research into improving IVF treatment. The major improvements sought are:

- an increase in the treatment's likelihood of success
- alleviation of the distress and risks endured by the woman, by means of improving the stimulation protocols and (in due course), bringing oocytes to maturation outside the woman's body *(in vitro* maturation)
- cryopreservation of oocytes with a view to reducing the number of surplus embryos produced as a result of IVF, as well as for the benefit of women who may become infertile owing to oncological treatment.

3.1 Improving the likelihood of IVF's success

Twenty years after the first IVF baby, the likelihood of an ongoing pregnancy is still not greater than approximately 15-20% per treatment cycle started (GR97a). Improving the likelihood is extremely important for involuntary childless couples who are dependent on this treatment. In the first place, given the same number of cycles, fewer couples would be left empty-handed. Apart from for IVF as a form of assisted reproduction, this will also certainly have consequences for the significance of PGD as an alternative for prenatal testing (chapter 2). Secondly, an increased likelihood of success per cycle would mean that women receiving IVF treatment would on average be less likely to need to undergo hormone stimulation and follicle puncture, and would therefore be less exposed to the associated risks and distress (GR97a).

In this section, the Committee provides a brief and by no means complete summary of the relevant research this entails, which is concerned with various parts of IVF treatment.

3.1.1 *Improving culture media*

In IVF treatment, fertilization and the embryo's subsequent development take place in a culture liquid. The optimum composition of this 'culture medium' is not known. Mimicking Fallopian tube fluid, in which fertilization and early embryonic development take place *in vivo,* has not been possible thus far. IVF in animals has shown that the composition of this fluid can be extremely embryo-toxic (Bor80). The compensation mechanisms for this that apparently exist in the Fallopian tube are still completely unknown.

One attempt to replace the culture media used for IVF involves the use of the so-called 'co-culture' (Wet95). In this method, somatic cells are added to the culture liquid that contains the embryos, with a view to improving the medium's culture properties. Examples include human granulocytes, human fibroblasts, bovine tubal epithelial cells and renal cells from the green African monkey (so-called 'Vero cells'). These added cells are supposed to produce substances that aid the early embryo's development, such as growth factors, or, alternatively, remove substances that could harm it. Co-culture is supposed to ensure that more embryos reach the blastocyst stage. There are safety hazards associated with the addition of cells, such as the transmission of viral and bacterial infectious diseases (Wet95).

Following its use with various species of animals, the step towards using co-culture with humans was taken at the end of the nineteen eighties (Bon89, Men90, Wie89). Although various centres reported the findings of research supporting co-culture's beneficial effect on the quality of embryos, not enough prospective randomized studies have been carried out as yet to investigate the effect on pregnancy. In one such study, researchers in Nijmegen were unable to find any difference in pregnancy percentages (Wet95). Co-culture was also used in combination with other techniques such as assisted hatching, cryopreservation and *in vitro* maturation (see below). As co-culture is also used in practically all research on *in vitro* maturation, it is difficult to determine how essential (or otherwise) the former technique is for the development of the latter. Many centres for bovine IVF have meanwhile abandoned co-culture and switched completely to synthetic culture media (Big97).

The fact that so little progress has been made in culture medium development appears to be explained by the exceptional properties of the (fertilized) oocyte in comparison with somatic cells. Moreover, each phase (fertilization, division, development) involves different requirements. In short, a great deal of fundamental

research still has to be carried out in this area (Dum97, Gar93a, Lee95, Sch96c, Ver95b).

3.1.2 *Selection of embryos by extended embryo culture*

In spite of the shortcomings of existing culture media, some centres are conducting research into the possibility of embryo culture for a longer period than is usual for IVF. Rather than being transferred on the second or third day, they are only transferred on the fourth or fifth day after insemination (Hui94, Sch96b). This obviously sets greater requirements for culture conditions in the laboratory. The idea is that embryos that continue to develop *in vitro* to the blastocyst stage have a greater chance of implantation than embryos that do not reach this stage *in vitro*. The advantage would be that a more efficient transfer policy can be adopted and that this would also lead to a reduction in the likelihood of multiple pregnancies.

However, it is not yet clear whether the benefits achieved using this method (in terms of each transferred embryo's chance of implantation) also translate into an increased likelihood of success for each cycle started. This raises the question of whether embryos that fail to develop, stop developing, or display fragmentation would have had a better chance of implantation if they had been transferred earlier.

3.1.3 *Improving the criteria for the selection of embryos for transfer*

An embryo's chance of implantation correlates with its development, as visible using a microscope. Examples of this are the division rate and division pattern, the absence of fragments, and compaction and blastocyst formation. A problem is that this correlation is less significant for the individual embryo. Researchers would like to have characteristics at their disposal that make it possible to also predict an individual embryo's chance of implantation more accurately and that can be obtained non-invasively. Research could for example be carried out into the substances secreted by the embryo or taken up from the culture medium. Some hormone-like substances may be important in the interaction between the mucous membrane of the womb and the embryo. Research into these markers is still at the fundamental stage (Gar93b, Lig97, Par96, Sim95, Sue97). It is still too early to assess whether routine determination of particular substances in the culture medium would be worthwhile in terms of improving the selection of embryos for transfer.

3.1.4 *Improving the embryo's chance of implantation.*

The preceding chapter mentioned a possible form of PGD that involves assessing embryos for chromosomal defects with a view to increasing the embryo's chance of implantation.

Besides, research into the value of'assisted hatching' is of particular importance in connection with this. Hatching is the process in which the embryo emerges from the oolemma. In assisted hatching the embryo is assisted by making an opening in the oolemma, usually by means of chemical agents. The assumption is that implantation sometimes fails to occur owing to the excessive hardness of the oolemma, which prevents the embryo from emerging under its own strength. The technique was first described in 1990 (Coh90).

There is controversy about the value of assisted hatching. Prospective randomized studies of assisted hatching in younger patients failed to show any effect on the chance of implantation (Hur98). According to the advocates of the technique, its significance should be sought particularly in a selected group of patients: older women and women for whose treatment embryos are used that have been frozen (Coh92, Sch94, Sch95). Insufficient data is available to assess this claim. A recently published randomized study failed to confirm it (Lan98). The study was only concerned with assisted hatching in older women.

3.1.5 *Embryo splitting to increase the number of embryos available for transfer*

As long as the cells (blastomeres) of which the early embryo consists are still capable of developing, either individually or together with one or more others, into a separate blastocyst, it is theoretically possible to make use of this capability to produce two or more embryos from a single fertilized oocyte. This is known as 'blastomere separation' or 'embryo splitting'. Success was recently achieved in cows and sheep using this technique to produce as many as four calves or lambs from a single oocyte. Although this could theoretically improve the effectiveness of cattle production, as yet it does not constitute a realistic option, owing to the large loss of embryos (Jon94).

In 1993, Hall, an American embryologist, reported having succeeded in splitting 17 human embryos in various stages of division and providing the individual cells with an artificial oolemma (Hal93). The purpose of the research was to determine the degree to which embryo splitting in the context of IVF could be used in humans. The advantage of this would be a doubling of the number of transferable embryos per fertilized oocyte. This would be particularly important for couples with a low yield of fertilized oocytes (Coh94). However, the assumption that embryo splitting can

increase their likelihood of success is contested on theoretical grounds (Jon94). Further research using animals and possibly human embryos may provide more clarity about this. The same applies to the possible harmful consequences for the offspring.

Whether or not the moral status of human embryos is an argument against splitting depends on the meaning ascribed to the concept. The Committee discusses the main positions in the debate about this in the next chapter. In this section, while referring to that discussion, the Committee simply notes that only those who believe the human embryo is a person (or should be considered as such) would consider preclinical research into embryo splitting as absolutely unacceptable, owing to the status of the embryo. Other moral considerations are concerned with bringing genetically identical people into the world as a possible consequence of transferring split embryos (CCN97, NAB94). This is not the aim of the procedure when embryo splitting is used to increase IVF's likelihood of success. However, it is a foreseeable consequence of doing so. The question is what this implies from the moral point of view*. It is generally not true to say that the production of genetically identical children constitutes an infringement of the principle of respect for human individuals. Identical twins are also born by virtue of a natural event, without any detriment to the individual uniqueness of the people concerned. Personal identity is determined by other factors in addition to genetic constitution. However, freezing embryos produced after splitting for use in future IVF attempts could result in genetically identical children being born after an interval of many years. The possible consequences of this certainly require further consideration (ASR97, NAB94). The danger of a 'slippery slope' has also been pointed out in this discussion (Mac94, McC94). Acceptance of embryo splitting as an aid in IVF could reduce the step in the direction of intentional 'genetic copying' of existing (or deceased) people.

Some members of the Committee expect so little from embryo splitting within the context of IVF that they consider further research undesirable, also bearing in mind the question of the technique's moral acceptability. Other members of the Committee have deferred reaching an opinion. They believe further preclinical research into the work and safety of embryo splitting need not necessarily be excluded but should be made dependent on the outcome of other considerations regarding the technique's acceptability (Wer95). The Committee agrees that clinical experiments with embryo

* In the discussion about this, embryo splitting is often represented as a type of cloning. Whether or not this is correct depends on how the term is defined. In scientific literature, cloning is usually understood as: asexual reproduction through the transfer of the entire genome (ASR97, Coh94). This does not apply in the case of embryo splitting. However, it is true that the possibility of bringing genetically identical people into the world through embryo splitting raises questions that are also raised in the discussion of cloning by means of cell nucleus transplantation (CCN97, NAB94).

splitting will not be acceptable under any circumstances until the safety aspects have been adequately examined preclinically.

3.2 Alleviation of the distress and risks endured by the woman

In the woman's natural cycle, a number of oocytes reach almost complete maturation every month. The follicles in which they occur increase in size considerably. However, one follicle is dominant and develops further than the others. As a rule, a single mature oocyte is released at the time of ovulation, which may be fertilized in the cycle concerned; the other follicles and the available oocytes they contain perish. Although IVF is sometimes used in the natural cycle (Day95), the availability of only one oocyte provides such a small chance of success that it is generally not considered a realistic option. It is therefore practically always preceded by hormone stimulation of the ovaries, to bring the oocytes in the non-dominant follicles to a sufficient level of maturity. The aim is to obtain eight to ten usable (mature) oocytes for IVF. Just before ovulation, they are aspirated from the follicles, under ultrasound guidance, via the vagina (follicle puncture). The actual IVF procedure can commence immediately afterwards.

Hormone stimulation is distressing for the woman and not without risks (GR97a). The Committee therefore underlines the importance of research into the possibility of reducing (by further improvement of the stimulation protocols) or completely eliminating the distress and risks (by maturing oocytes outside the body).

3.2.1 Further improvement of stimulation protocols

Increasingly more complex stimulation protocols have been developed to achieve better results. However, an unwanted consequence of attempting to maximize the number of mature oocytes obtainable per cycle is an increase in the risk of sometimes severe complications, such as ovarian hyperstimulation syndrome (OHSS). The aim of research in this field is to achieve simplification of the stimulation protocols, with fewer risks for the woman, while maintaining more or less the same likelihood of success. To achieve this, rather than focusing on maximization, the hormone treatment will have to focus on optimizing the results. Work is underway to develop a new generation of medicines (GnRH antagonists) to prevent disrupted follicle growth in the IVF cycle. It is hoped that this will reduce the negative side-effects, while reducing the length and intensity of the stimulation of the ovaries (Fau98).

Research focusing on improved possibilities for predicting how individual patients will respond to stimulation is also important (Jan97). Being able to adjust the

administration of the hormones to the individual patient's characteristics (which is not currently possible) could result in a further reduction in the risk of OHSS.

Finally, the Committee refers to research into the value of 'recombinant' preparations of various compositions (Cha95). It is assumed that their use could lead to better quality embryos (and consequently a greater likelihood of success). It is not yet possible to say whether this assumption is correct on the basis of the data available at present.

The Committee drew attention to the possible negative side-effects of hormone stimulation in its previous advisory report (GR97a). That aspect should also be taken into account in research into improving the current protocols and preparations (Cah97, Dun90).

3.2.2 *In vitro maturation oocytes (IVM)*

Completely abandoning the current use of hormone stimulation (without reducing the likelihood of success) would represent a considerable improvement in IVF treatment. With a view to achieving this, research is underway into the possibility of cultivating immature oocytes (obtained in an unstimulated cycle) outside the woman's body until they are sufficiently mature to be fertilized. This procedure is known as *in vitro* maturation oocytes (hereinafter referred to as IVM).

If IVM could replace the customary use of hormone stimulation, besides benefiting women who wish to undergo IVF treatment using their own oocytes, it could also benefit women who are dependent on oocyte donation. If, for oocyte donation, immature oocytes could also be used, expectations are that more women would be prepared to provide oocytes. The existing shortage of donor oocytes both for assisted reproduction and for scientific research could then be reduced.

Effectiveness

IVM has long been the subject of animal-based experimental research (Epp77, Epp96). It has been demonstrated for various species of mammals that it is possible to enable oocytes taken from removed ovarian tissue to mature *in vitro,* to fertilize them and thus produce offspring (Epp89, Gil97, Mer96). Although different for each species, the yield is, however, low: many, if not very many, oocytes/embryos are lost during each new stage of the procedure (maturation, fertilization, embryonic development) (Nay94). The best results appear to be achieved using oocytes taken from the largest follicles which are therefore already the most mature *in vivo* (Mer96). This indicates that the conditions under which oocytes reach maturity in the body can still only be inadequately synthesized in the laboratory. Research is therefore mainly concerned

with improving laboratory conditions (Ala96, Lon96), as well as obtaining an insight into the factors that determine the development potential of immature oocytes (Aml96).

It is also possible to bring human oocytes to maturity *in vitro*. Clinical experiments in centres outside the Netherlands (involving the transfer of embryos into the womb following IVM) have resulted in the birth of several children since the early eighties (Cha91, Edi97, Jar97, Nag96, Rus97, Tro94, Vee85). However, taken as a whole, the results are disappointing. Only two percent of the women from whom immature oocytes were taken with the hope of transferring an embryo went home with a child (Tro96). It has to be pointed out here that IVM is mainly used on oocytes that possibly already had a less favourable starting position *in vivo*. Some studies (including the pioneering research by Veeck) were concerned with 'saving' oocytes that proved to be insufficiently mature for IVF after hormone stimulation and aspiration (Nag96, Vee85). Most other research was concerned with oocytes from women with polycystic ovaries (Bar95, Tro94). This common condition (polycystic ovary disease: PCOD) among women with fertility problems means that the growth of the follicles in the ovaries comes to a halt. Instead of developing into a mature follicle of the normal size of approximately 1.5 to 2.5 centimetres across, the development of the follicle stops at around 0.8 to 1 centimetre in women with polycystic ovaries. The number of follicles is much larger than normal, which means that a relatively large number of immature oocytes can be obtained per puncture. The expectation based on this — that IVM would offer these women a greater chance of pregnancy — failed to materialize, probably because their oocytes are generally of an inferior quality (Tro94).

Whether IVM will in future enable the replacement of standard hormone stimulation in IVF treatment depends on the results obtained with normally ovulating women; who after all form the largest group of IVF patients. Very little research has been conducted into this thus far. The results appear to indicate that better results are achievable among normally ovulating women (per oocyte obtained) than among women with ovulation disorders, including PCOD patients (Bar96).

A considerable improvement in the fertilization percentage after IVM seems to be possible if ICSI is used with fertilization, instead of normal IVF (Bar95, Hwa97, Nag96). The explanation for this appears to be that, as a result of the oocyte's period in suboptimal culture conditions outside the woman's body, the oolemma *(zona pellucida)* may undergo changes that make it impenetrable to sperm cells (zona hardening), something that normally only occurs after a single sperm cell has penetrated (Bar95). In ICSI, a sperm cell is injected into the oocyte's cytoplasm; using this method means that premature hardening of the *zona* cannot prevent fertilization. However, the most critical phase is not so much fertilization but the development of the early embryo. Fertilized IVM oocytes, also those of normally ovulating women,

appear to display blocked cell division more often than is the case after *in vivo* maturation (Bar96). The explanation for this is sought in a possible retardation of the development of the oocyte's cytoplasm, owing to inadequate culture conditions *in vitro.*

Safety

IVM was introduced into cattle farming as part of an entire *in vitro* and thereby more readily managed embryo production process (IVP) (Tho97). Many calves and lambs have since been born from embryos produced *in vitro.* This method is not very efficient in comparison with the normal procedure used in this sector (i.e. *in vivo* production followed by uterine lavage to remove the embryos from the womb) (Tho96, Wri95). This need not be a problem, provided sufficient oocytes are obtained for IVP. It provides major operational advantages. More embryos can be produced in the same time and with lower risks.

However, the results also indicate possible negative health impacts. Various publications show that using IVP in cows and sheep leads to a higher average birth weight, a larger perinatal mortality rate, disruption of the sex ratio (more male offspring) and a higher average percentage of congenital abnormalities (Beh95, Hol96, Kru97, Wag98)*. The abnormalities (in organs, the spine, limbs and joints) indicate disturbed foetal development. It is assumed that this disturbance is the result of changes in the regulation of early gene expression, caused by the conditions under which embryos are produced *in vitro* (Kru97, Tho97, Wal96).

IVP consists of three parts: IVM, IVF and IVC *(in vitro* culture: culture of embryos to the blastocyst stage). The question concerns which of these parts has to be linked to the aforementioned phenomena (also known as 'large calf syndrome'). Some authors believe the cause should mainly be sought in the composition of culture media (Tho97, Wal96). On the other hand, there are the results of a study of IVP in sheep, in which the development of some of the embryos obtained following IVM was continued *in vivo* to the blastocyst stage (in a surrogate mother sheep) rather than *in vitro* (Hol96). Higher birth weights were found in both cases vis-a-vis the control group of lambs produced entirely *in vivo.* The researchers concluded that the different findings were also in any case linked to the use of IVM and IVF in the production of embryos.

* The last of the publications mentioned concerns a prospective comparative study recently carried out in the Netherlands of several thousand calves, some of which came from embryos produced *in vitro* and some *in vivo.* The percentage of congenital abnormalities (hydro-allantois, spinal and limb abnormalities) in these groups were 3.2% and 0.7% respectively. The IVP calves were an average of 4 to 5 kilos heavier, a difference of 10%.

Theoretical grounds have also been put forward for a possible relationship between IVM and the occurrence of developmental defects (Tes96). The defects may be the result of a disturbance of the process of'genomic imprinting' (Lat95). The factors that regulate this process may not have developed completely, owing to the use of immature gametes (or gametes matured under inadequate conditions).

The Committee believes further clinical experiments with IVM should be halted until more clarity is obtained in preclinical research about the technique's safety (Wer93). Besides focusing on possible chromosomal damage, any such preclinical research should be specifically concerned with the question of whether IVM can lead to a disturbance of early gene expression and, if it can, what the consequences may be for the offspring.

3.3 Oocyte cryopreservation

IVF treatment could be further improved by the possibility of freezing oocytes for later use. In the first place, it would no longer be necessary to produce embryos from all the oocytes obtained after hormone stimulation (or IVM). At present, if there are many more oocytes than the number of embryos that can responsibly be transferred in a single attempt, the options are to either produce and freeze 'supernumerary embryos' or to allow the valuable oocytes to perish. In view of the likelihood of success and the minimization of the distress and risks for the woman, the former option is practically always chosen (GR97a). An important disadvantage is that supernumerary embryos can end up as surplus embryos. This could largely be avoided if oocytes could be frozen. It would then only be necessary in each treatment cycle to fertilize the number of oocytes needed to obtain the maximum number (two or three) of embryos that can responsibly be transferred at one time. Because this necessitates taking into account the possibility of a failure in the fertilization attempt, or of it resulting in embryos of an inferior quality for transfer, more embryos would on balance always be produced than the number transferred. However, the difference in number would be smaller than it is at present. Those who consider the production of surplus embryos for IVF treatment acceptable will also see this as a moral gain.

Secondly, the possibility of freezing oocytes could be of major importance for women who face the prospect of becoming infertile early in life as a result of oncological treatment. Women in this situation could have their oocytes frozen to ensure the possibility of being able to have children at a later date. This is referred to as 'fertility insurance'*.

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* These women are not ensuring the restoration of their own fertility but the possibility of undergoing IVF treatment. Given IVF's likelihood of success, the possibility of this leading to the birth of a child remains questionable. An approach intended to remove this shortcoming has been proposed by the British researcher, Gosden (New96, Okt98). In

Finally, the possibility of freezing oocytes would provide benefits for couples who are dependent on IVF using donor oocytes. Oocyte cell banks similar to existing sperm banks could be set up. This is assuming the availability of IVM as an alternative for hormone stimulation and the resultant anticipated reduction in the current shortage of donor oocytes. An additional advantage would be that freezing donor oocytes would create the possibility, as is normal in the case of sperm donation, of including a quarantine period, after which the donor is tested again for HIV, in order to completely exclude the possibility of contamination.

Effectiveness and safety

In the second half of the nineteen eighties, a number of researchers transferred embryos produced from frozen and subsequently thawed human oocytes (A1H86, Che86, Ueh87). The results were disappointing: in total five pregnancies were achieved from more than 900 oocytes (Kar96). The reason for the lack of success is being sought in chromosomal damage as a result of a disturbance of the internal organization of the oocyte, which is in a vulnerable stage at the time the puncture is made (just before ovulation) (Bou92, Els93).

The disappointing results of the first clinical applications led to experiments of this kind being almost completely discontinued during the following ten years. Research mainly focused on obtaining a better insight into the underlying biological mechanisms. An attempt is now being made on the basis of the knowledge thus obtained to improve the cryopreservation protocol in order to prevent chromosomal damage (Ber96, Goo94, Kar96, Mar96).

However, other researchers believe a new way has to be sought (Cor96, Okt98). In the approach they favour, instead of mature oocytes, immature oocytes are the chosen starting point. It is biologically plausible that they are far less sensitive to damage as a result of freezing and thawing. However, freezing immature oocytes is only worthwhile if they can be subsequently matured *in vitro*. As explained in the preceding paragraph, that is not yet a realistic possibility.

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studies using mice and sheep, he showed that restoration of ovarian function is possible by 'autografting' pieces of ovarian tissue that have been excised and cryopreserved (Gos94). Around 20 women who required oncological treatment have since had pieces of ovarian tissue frozen, in the hope that it can be subsequently grafted back (Bah96). The stated advantage of this approach is that these women, once recovered, will be able to have children in the normal way. IVF would not be necessary. On the other hand, however, there is a concern that this approach involves a risk, precisely because it relates to women with cancer. Any cancer cells in the transplant could result in the woman who has just recovered contracting cancer again. (Sha96). The question is whether this risk can be covered by careful patient selection (as for some types of cancer there appears to be no risk of the cancer returning in this way), or by random testing of the removed tissue for the presence of cancer cells (Gos97, Sha97). There is no risk of transmitting the old disease in the case of frozen oocytes.

Research into the cryopreservation of immature oocytes has been conducted on human oocytes (only in preclinical research), besides oocytes taken from animals (Can94, Cor96). Researchers in the United States found no difference in the developmental capacity (as shown by the results of IVM) of frozen and subsequently thawed immature oocytes vis-a-vis a control group of oocytes that had not been frozen (Tot94a). Likewise, in a follow-up study in which oocytes were also fertilized, no differences were found in the fertilization percentage, nor in the percentage of fertilized oocytes that displayed normal embryonic development (Tot94b). However, the latter results do not correspond with the findings of a Korean research group (Son96). These researchers also found an increased percentage of chromosomal and structural abnormalities in oocytes matured *in vitro* after having been frozen (Par97).

The Committee believes further clinical experiments into the possibility of freezing and storing oocytes can only be conducted responsibly if more clarity is obtained in preclinical research about the safety of the technique. The main question in any such research should be concerned with possible chromosomal damage. The issue of whether it is responsible to conduct clinical research into the cryopreservation of immature oocytes should be assessed in combination with the issue of the safety of IVM (see 3.2.2).

3.4 New possibilities, new questions

The Committee underlines once more the importance (including the moral importance) of research concerned with improving IVF as a form of assisted reproduction. The Committee believes in particular that the availability of IVM would represent a qualitative improvement. In the case of a specific group of patients in need of fertility insurance, this would also certainly apply to the possibility of freezing oocytes. Another matter of moral importance is the reduction the latter technique could bring about in the number of surplus embryos produced after IVF.

However, making both of these techniques available in clinical practice would also raise new questions, each of which would require further consideration. The Committee only touches on these questions here.

3.4.1 Donation of immature oocytes or ovarian tissue: the Organ Donation Act

In the first place, this is concerned with questions about the aforementioned significance these techniques (certainly in combination) could have for reducing the current shortage of donor oocytes. Immature oocytes can be obtained through follicle puncture (under ultrasound guidance, via the vagina), or through ovarian tissue biopsies from patients undergoing sterilization. In connection with this, the Committee

calls for attention to be paid to the applicability of the Organ Donation Act (Stb96). It points out that gamete donation is not covered by the Act (section 1, under b), whereas it may cover the provision of ovarian tissue, as a source of immature oocytes.

3.4.2 *Ovarian tissue from deceased women or foetuses as a source of oocytes*

Immature oocytes could, however, also be obtained from the ovarian tissue of deceased women *(post mortem* donation) or even from aborted foetuses (Har96c, HFE94). In determining whether these are acceptable possibilities, it is necessary to distinguish between donation for scientific research and donation for assisted reproduction. In the latter case, the interests of the resultant child must be paramount. It is not inconceivable that the child's emotional development could be harmed by the knowledge that it was conceived using cell material from a deceased person (or even an aborted foetus). It is therefore necessary to ask whether such uses of cell material are acceptable.

3.4.3 Fertility insurance for non-medical reasons

The possibility was mentioned above that freezing oocytes may be of major importance for women who are at risk of becoming infertile as the result of oncological treatment. This approach would enable them to keep the option of undergoing future IVF treatment using their own oocytes. If this could be carried out using immature oocytes (which also assumes the possibility of IVM), oocytes could be frozen as a form of fertility insurance, without the woman first having to undergo hormone stimulation.

If fertility insurance only requires the freezing of immature oocytes, the issue arises of this technique's application for non-medical reasons. It is conceivable that this would be requested by women who wish to postpone having children until a time in their lives when it might no longer be possible (without problems) by natural means. Oocytes are already frozen in the United States for this reason. It is questionable whether this is a desirable development. Furthermore, the Committee refers to the arguments put forward in its previous advisory report for an age limit for IVF using 'younger' oocytes (GR97a).

3.5 Importance of preclinical research

The Committee notes that new methods and techniques in artificial reproduction are sometimes tested in clinical practice without adequate preliminary studies using animals having been conducted to determine any harmful effects, if possible also in the

long term and over several generations. The Committee considers this irresponsible, as it could mean that women and children are being exposed to avoidable health risks.

The finding that no harmful effects occur in animals does not anyway automatically justify the conclusion that a new technique can be used safely in humans (Bur95b). A step in the dark is always involved to some degree, owing to the possibility of species-specific differences. When new methods or techniques in artificial reproduction are involved (as discussed in this as well as the previous chapter), it is often only possible to say whether the step to the clinic is responsible after preclinical research using human embryos has been conducted (i.e. without subsequently transferring those embryos). The usefulness of any such research depends on whether it could lead to a reduction in the uncertainty remaining after animal research, concerning the safety of the technique, that would be relevant for the further policy. Although it is certainly not possible to clarify all safety aspects in preclinical embryo research, it would be possible to examine the potential for development (fertilization, division, blastocyst formation) and the chromosomal constitution of embryos produced after the application of a new technique (AFS86, Tro90). Considering that a large percentage of the embryos produced in normal IVF practice cannot be cultured to the blastocyst stage, and because approximately half of the embryos produced using normal IVF have chromosomal aberrations, it is necessary to determine beforehand which findings would lead to a different conclusion for the future policy. Depending on this, it would then be possible to determine how many embryos are required for research. Concrete research proposals would have to be tested against these methodological aspects.

The above does not say whether or not it is acceptable to use human embryos for scientific research. The Committee discusses this in more detail in the next chapter.

Chapter 4

Research using human embryos

4.1 Introduction

In this chapter the Committee examines the discussion of the acceptability of research using human embryos *in vitro*. Whether and under which circumstances research is allowed using embryos *in vivo* is an entirely different issue that is beyond the scope of this advisory report. Moreover, the discussion here is limited to research involving embryos that are not intended to be transferred; this is referred to as 'consuming' embryo research. Research involving embryos that are to be transferred is only responsible if there is no risk of harmful consequences. The research must not jeopardize the health of any child resulting from the transfer.

The most important aims of research conducted thus far *(consuming embryo research)* are (Mau96, McL96, NIH94):

- to increase knowledge of the causes of diminished fertility
- to improve treatment possibilities for diminished fertility
- to develop methods for determining genetic or chromosomal abnormalities when selecting embryos for transfer
- to develop new methods of contraception.

The promising fields of research mentioned include (GR97b, McL96, NIH94):

- increasing knowledge of the causes of congenital abnormalities
- developing therapeutic possibilities concerned with the healthy development of human embryos

- increasing knowledge of the factors involved in the origins of types of cancer
- research using embryonic stem cells, with a view to using them as a source of transplant material.

Not all these fields of research are covered by this chapter. In accordance with its assignment, the Committee wishes to limit itself to the issue of the acceptability of using human embryos for the research referred to in the preceding chapters of this advisory report: i.e. the further development of PGD and the improvement of IVF.

4.2 Ethical aspects

4.2.1 Three views of the moral status of the embryo in vitro

Each position taken in the discussion of the acceptability of research using human embryos *in vitro* assumes an opinion on 'the moral status' of human embryos *in vitro*. Having a moral status means: (to a greater or lesser extent) being worthy of moral consideration (Her96). The moral status of human beings is connected with their personhood. As persons, they deserve respect and, where necessary, protection. The question is whether and to what degree human embryos *in vitro* are also worthy of protection. The answers provided to this question can be divided into three groups, according to the underlying view of the relationship between the moral status of human beings and that of human embryos *in vitro*.

Equally worthy of protection

According to some, the moral status humans have as humans (persons) accrues also and to the same degree to human embryos *in vitro* (Eij97, Hon96, Joc89, LIN88). According to this view, the embryo is no less worthy of protection than a child or an adult human being and should be treated accordingly.

A variant of this view is based on the concept of personhood as used in Roman Catholic teachings. According to that concept, personhood is seen as given, not with any human faculty, but with the essence of humanity as it appears in the unity of body and soul (Boy91, Don87). Although this view leaves space for uncertainty about the embryo's ontological status (whether or not it is a person) at any time during its development, this does not constitute moral leeway: we cannot risk killing a person (Hon96).

Another variant assumes the 'modern' concept of personhood, which is defined in terms of certain human faculties (which in any case include a form of self-awareness) (Kob97, Loc64). According to this view, the fact that human embryos do not fulfil

criteria for personhood given with that definition is not conclusive. Conclusive is that they can be considered as 'potential persons'. Potential persons would have the same moral status as persons and would therefore be worthy of the same degree of protection (Rei97, Ved93).

Not worthy of protection (but with a symbolic value)

Others believe that human embryos *in vitro* have no moral status that makes them worthy of protection. They do not regard the capability of developing into a human being as the actual purpose of an embryo but only as a possibility, which moreover depends on whether or not it will be transferred (Har85, Har93a, Sin90). According to these authors, there is no reason to treat the embryo *in vitro* differently from the gametes from which it emerges.

Having 'no moral status' need not mean that human embryos *in vitro* may be subjected to every form of treatment. This may be opposed for other reasons, including the 'symbolic value' that human embryos (a beginning form of human life) actually have in our society (Har93a, Rob86, Ste92). So conceived, it is not for the sake of the embryo itself that it ought to be spared but for the sake of the community in which it has acquired social significance (Har93a, Rob95).

Relative worthiness of protection

A characteristic of a third group of views is that moral relevance is attached to both the difference (the embryo is not a human being) and the continuity (a human being could develop from the embryo). This represents a position between the two views discussed above. On the grounds of its human origin and its potential to develop into a human being, the embryo *in vitro* has an intrinsic value according to this understanding and therefore is worthy of protection. On the other hand, it is stressed that the embryo's moral status is not equal to that of human beings. The embryo's worthiness of protection is therefore regarded as relatively limited (Her96, Kui91, Rei93, Wer89). Often (not always, see for example Rei93) this is augmented with the notion that the moral status of human embryos and foetuses increases with their development (Her96). Proponents of this position speak of'increasing worthiness of protection' (Dup88, Wer89). In different variants of this gradualistic view, various 'moments' in embryonic (or foetal) development are referred to as a transition to a stage in which the embryo would be more worthy of protection.

4.2.2 *Research using human embryos in vitro*

Only those adopting the first position described above ('equally worthy of protection') would reject consuming embryo research absolutely, regardless of how important any such research might be (Joc89). The other two positions have in common that embryo research is not deemed morally unacceptable in advance. However, insofar as the embryo is attributed a certain value (worthiness of protection on the grounds of its own moral status or a symbolic value), any form of research using human embryos requires further justification. The fact that it might lead to greater knowledge or that useful applications are conceivable is not sufficient; it has to be clear that the interests served by the research are also morally more important than the value attributed to the embryo *in vitro.* This position regarding the justification of consuming embryo research is adopted in practically every report published by committees both in the Netherlands and elsewhere on this subject (CCN86, GR86, GR89, NIH94, RCN93, War84).

4.2.3 Origin of embryos for research

When room is allowed on the basis of this viewpoint for consuming embryo research, the condition is sometimes attached that human embryos must not be specially produced for that purpose. Only surplus embryos from IVF practice may then be used for scientific research. This means that the research that cannot be carried out with these cannot take place, regardless of what the importance of any such research might be (Eis97, GR86, Kui89, Spr89).

Surplus embryos are the embryos that are not transferred to the woman for whose treatment they were produced. The majority of these are embryos that, for reasons of quality, are not suitable for cryopreservation. In the unusual case of it being decided in the IVF treatment not to freeze any embryos, all embryos not transferred in the cycle in which they were produced are 'surplus embryos'. Embryos that have been frozen for any length of time are only classified as surplus embryos when the woman (the couple) has indicated to renounce having them transferred in any subsequent cycle or treatment. Finally, there are also the embryos that are produced from an oocyte that is fertilized abnormally (for example by two sperm cells) and for that reason should not be transferred.

The possibility that 'surplus embryos' might in practice be produced for research purposes has been pointed out (War84, Wer91). Such would be the case if a more intensive stimulation regime were to be undertaken than was necessary for the

woman's treatment. This is obviously unacceptable. The fact that it is possible brings into question the practical enforceability of the 'only surplus embryos' condition.

An argument for prohibiting the production of embryos for research would be that special production completely 'instrumentalizes' the embryo, unlike in the case of surplus embryos, which are produced with the intention of transferring them (Bur95a, CCN86, GR86, Kui89). As is clear from the latter part of this wording, the argument is not so much concerned with the nature of the use of the embryo, which is instrumentalising in both cases (Bea96), as with the reason for the embryo's production. In one case (surplus embryos), this is with the intention of allowing the embryo to develop into a child. As this is no longer a realistic possibility (otherwise there would be no such thing as a surplus embryo), the choice is between allowing the embryo to perish or (first) using it for scientific research. In the other case, an embryo is produced exclusively for use in scientific research (Kui89, Wer89). This makes no difference to the embryo's status (Ger93). However, it does raise the question of whether it is morally acceptable to produce embryos exclusively for use in scientific research. Besides this difference in intention, there is another morally relevant difference: surplus embryos are bound to be lost anyway, whereas producing embryos for research leads to embryos being lost that would otherwise not have existed.

Recognizing the relevance of these considerations need not mean that they have to be viewed as decisive. Various committees and authors have argued that any such recognition does not (decisively) affect the outcome of the balanced reasoning referred to above (KEM91, NIH94, Rei93, Rob94, War84, Wer87, Wer89, Wer91).

Whether producing embryos for research is acceptable also depends on whether the oocytes required for this can be obtained in a morally responsible manner. Is it acceptable in aid of scientific research to ask women to undergo treatment (hormone stimulation and follicle puncture) that involves a risk to their own health (GR97a)? Is it acceptable in aid of scientific research to ask a woman undergoing IVF treatment to relinquish some of her 'harvested' oocytes and thereby reduce the likelihood of her IVF treatment being successful?

4.2.4 Maximum development period

The doctrine of'increasing worthiness of protection' implies that at some 'moment' in the development of the embryo (or foetus) it is no longer acceptable to use it for scientific research*. But those who wish to allow leeway for embryo research on grounds other than 'increasing worthiness of protection' are equally faced with the

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* The word 'moment' is in inverted commas to indicate that qualitative changes in the biological development occur not in stages but by virtue of processes for which it is only possible to give average time indications.

issue of having to decide how far embryonic development should be allowed to proceed within the scope of research.

As in the British Warnock report, the *terminus ad quem* for embryo research is often placed at a development period of 14 days; this corresponds with the 'moment' at which most embryos developing *in vivo* have completed the process of implantation in the womb (nidation) (War84). Various arguments are put forward for adopting this limit (Boe93, RCN93). One argument is that around this time the cells from which the future individual will develop become visible as an organized unit. It is only then that there is no longer a possibility of identical twins being produced by a split in the embryo. A fixed individual identity cannot be attributed to the embryo until this 'individuation' has occurred. Another argument that is often cited is that in the organization of cells that exist at around this time, a precursor develops of what will eventually become the central nervous system: the 'primitive streak' (NIH94).

Others contest the moral relevance of these events around the fourteenth day and take as the point of departure the 'moment' at which the first brain activity can be demonstrated. This argument takes the position that the embryo fails to satisfy the minimum characteristic of personal identity before this, namely consciousness (Loc88, Sin84, Tau85, Wer87). This would place the limit at around six weeks. Because the development of brain functions extends over a long period, it is not surprising that even later limits have been proposed, on the grounds of a less basic interpretation of the criterion of brain activity (which is sometimes compared with that of brain death at the end of life)(Jon89).

The long culture of human embryos *in vitro* is anyway not an issue with the current level of knowledge. After the seventh day, development clearly changes from normal development in the womb; a type of unorganized, 'cancerous', growth pattern arises (GR92).

4.3 Legal aspects

The human embryo *in vitro* is not a person in the eyes of the law and there is also no legal argument for treating it as such. Human beings only become part of the legal community through birth (Bro96). However, this is not to say that the unborn enjoy no legal protection whatsoever (GR89). The accepted view is that current law provides sufficient starting points for a legal theory in which the human embryo is allowed an increasing level of protection, according to its phase of development (Bra89, Bra95, Lee94).

4.3.1 *Progressive legal protection*

The 'theory of progressive legal protection' is the legal pendant of the ethical position indicated above as the 'doctrine of increasing worthiness of protection'. The main starting points for this legal theory are found in the Pregnancy Termination Act. The following standards are derived from the Act:

- pregnancy termination is not (any longer) permitted if the foetus is independently viable
- the abortion of a not independently viable foetus is only permitted if this is unavoidable because of an emergency situation on the part of the woman (and taking into account the other provisions of the Pregnancy Termination Act)
- the provisions of the Pregnancy Termination Act do not affect the use of agents to prevent the embryo's implantation in the womb.

According to the accepted medico-legal interpretation, these provisions mean that first the implantation and subsequently the viability limit can be designated as 'marker points' in the development of the human embryo, to which the law has attached the consequences of a greater degree of protection (Bra93). The development is thus devided into three phases:

- from fertilization until the embryo's implantation in the womb (completion of nidation, 14 days after fertilization)
- from implantation until the foetus is independently viable (pregnancy duration of around 24 weeks)
- from the viability limit to birth.

Leenen describes implantation as the transition between the *status potentialis* and the *status nascendi.* After implantation, the birth of a child can only be prevented if the embryo's growth and development is interrupted for any reason; prior to this, the embryo has the potential to develop into a child, but something else has to occur before it can realize this potential (Lee94). An implanted embryo enjoys a degree of legal protection* that allows no scope for scientific research. However, this does not apply to an embryo that still has the *status potentialis.* Leenen says of this: "The emergence of the human pre-embryo from human gametes and its potential to grow

* For the legal relevance of the moment of implantation as the moment of transition in the theory of progressive legal protection, besides referring to the Pregnancy Termination Act, Leenen also refers to section 2, Book 1, of the ' Netherlands Civil Code. This stipulates that "the child with which a woman is pregnant is already considered as having been born whenever this is demanded by its interests". There is no question of pregnancy until the embryo's implantation in the womb.

into a human being call for caution in its handling, but the worthiness of protection is limited, owing to the indeterminateness of the pre-embryo and the primitive phase of its development". lts use for scientific research is therefore permissible according to proponents of the theory in question, provided certain criteria are observed. The scope this creates also permits research using embryos that have been produced especially for that purpose. In terms of the status the law ascribes to the embryo, it makes no difference what the intention was in producing it (Bra89, Lee94, Sut93).

There is greater agreement among medical lawyers about the *terminus ad quem* than in the ethical literature on embryo research. The starting point is that the embryo's legal status is not affected by whether it is *in vivo* or *in vitro.* The upper limit has to be set at a development period of 14 days. After that period, an embryo developing *in vivo* would have implanted, and its worthiness of protection would then be of the higher level associated with the *status nascendi.* By analogy, this status change therefore also applies to an embryo cultured for longer than 14 days *in vitro* (Bra89, Lee94).

However, the consensus described above is certainly not complete. According to Sutorius, the *status nascendi* only begins at the viability limit, and there can be no legal protection before then (Sut93). According to Van der Burg, current law does not provide a starting point for the position that the embryo *in vitro* is already to some degree (regardless of how small) worthy of protection (Bur94, Bur95a). There may well be moral reasons for treating it with respect. From the legal point of view, however, the embryo *in vitro* is deprived of any protection, even when cultured for more than 14 days. Van der Burg otherwise calls for legislation to be introduced as soon as possible to bring this, in his view, undesirable situation to an end. This is on the grounds of moral considerations that do not assume the (ethical) concept of increasing worthiness of protection, but which may be explained in terms of respect for the symbolic value of human embryos (Bur95a).

4.3.2 Future legislation

The fact that legislation will be introduced in this field was decided long ago. A bill (from 1992) which has since been withdrawn only left scope for 'therapeutic embryo research' concerned with the healthy development of the embryo, leading to pregnancy (Har93b)*. In March 1995, the present Minister of Health, Welfare and Sport, sent a memorandum to the Lower House also on behalf of the Minister of Justice, containing an outline of new legislation allowing scope for consuming embryo research, under strict conditions (TK95a).

* An exception was made for non-therapeutic research using polyploid embryos (which occur when an oocyte is fertilized by several sperm cells; embryos that are not suitable for transfer).

Article 18 of the European Convention on Human Rights and Biomedicine*, which will be put before the parliaments of the member states for ratification, stipulates that:

- where the law allows research on embryos in vitro, it shall ensure adequate protection of the embryo
- the creation of human embryos for research purposes is prohibited.

A separate Act will have to be introduced for the Convention's ratification in the Netherlands. The national legislation in countries that have adopted the convention must not conflict with the Convention's text. However, it is possible to make reservations in respect of certain articles. The United Kingdom is expected to do so in respect of the prohibition on the creation of human embryos for research purposes. A lot of research is conducted in the United Kingdom that would be affected by any such prohibition.

4.4 The Committee's standpoint

Following on from previous Health Council recommendations (GR86, GR89) and concurring with the aforementioned reports from other countries (CCN86, NIH94, RCN93, War84), the Committee believes that it may be morally and legally acceptable to use human embryos for scientific research, provided it involves a major health issue and due consideration is given to the interests of all those concerned.

4.4.1 Justification of research using human embryos

The issue of which view of the embryo's moral status is correct cannot be decided on the basis of independent arguments - arguments which owe nothing to one of the positions concerned in the discussion (Glo77). However, the first of the aforementioned viewpoints, i.e., that a person exists from the moment of fertilization (or in any case a being exists that should be treated as a person), is not compatible with the moral and legal scope permitted in our society for pregnancy termination, contraception using an intra-uterine device, and the acceptance of surplus embryos as a consequence of IVF (Joc89).

The Committee's standpoint is that while a given value should be assigned to the embryo *in vitro,* by virtue of which it deserves respect, this value is relative and can be overridden when other, also morally more imperative interests are involved. This leaves open the question of whether the value assigned to the embryo is based on its

* Convention for the protection of human rights and dignity of the human being with regard to the application of biology and medicine (CON96).

own moral status (worthiness of protection) or on any social significance it is assigned (symbolic value).

This view implies that embryo research is only acceptable on the basis of an appraisal of the relative importance of the value of the embryo *in vitro,* on the one hand, and, on the other, the moral importance of the proposed research. The Committee believes that allowing scope for research using human embryos can be justified on the basis of a balanced appraisal of this kind. The greater evil would be to abandon embryo research concerned with major human health issues. Within the context of this advisory report, this refers to the health issues affecting IVF patients and the children brought into the world through artificial reproduction.

This standpoint on the justification of embryo research immediately entails adopting a number of criteria any such research has to satisfy (Dav95, NIH94, RCN93, TK95a). In the first place, it must be clear that the research involves a major health issue. The greater the doubt about this, the more difficult it becomes to justify using human embryos for the research in question. The Committee stresses that this does not only concern the importance of the field of research but also the question of whether the proposed research could add something to existing knowledge in that field. Secondly, the setup and the execution of the research must be scientifically sound. Thirdly, it must not be possible to obtain the envisaged results in any other way (through animal research or cell culture, for example). Finally, the research must not use more embryos than is strictly scientifically necessary.

4.4.2 Research using embryos created for that purpose

The Committee has considered in detail whether it may be permissible to create human embryos for research purposes*. The Committee's answer is a qualified yes. It does not contest that there is a moral difference between designating surplus embryos for scientific research and creating embryos for research purposes (see 4.2.3). The Committee believes the value (worthiness of protection or symbolic value) that has to be ascribed to human embryos *in vitro* is a *(prima facie)* reason for not creating them simply as research material.

* The following standpoints have been adopted in previous advisory reports by Health Council Committees on the creation of embryos for research. In the 1986 advisory report on artificial reproduction, creating embryos specially for research was deemed morally unacceptable. One Committee member (while referring to the importance of the research this excluded) had a note added indicating the view that this was considered imprudent (GR86). The advisory report Erfelijkheid: wetenschap en maatschappij (Heredity: Society and Science), which was published three years later, specifically left open the question of the permissibility of creating embryos for research, also because the Committee was unable to reach consensus (GR89). Finally, in 1994, in its comments published on the draft European Convention on Human Rights and Biomedicine, the Standing Committee on Medical Ethics and Health Law considered the prohibition (see above 4.3.2) as rather unfortunate, owing to the ongoing discussion about its desirability (GR94b).

Although the use of surplus embryos is preferable on moral grounds, the Committee does not believe this means it is only justifiable to use surplus embryos. Such justification is after all based on the view that the embryo only has a limited value and that the moral requirement to give that value the greatest possible respect may be overridden by the possibly greater moral weight of the interests served by the scientific research in question. If the research cannot be conducted using surplus embryos and its importance for human health is beyond doubt, the Committee believes the use of embryos specially created for that purpose is justified, subject to the required oocytes being obtained in a morally sound manner (see below: 4.4.3).

The Committee does *not* consider the creation of research embryos acceptable for research that can be conducted using surplus embryos. This is the case for many research proposals but not for all (NIH94). Research may focus on processes, such as fertilization or the fertilized oocyte's stages of cell division, which have already taken place in surplus embryos. They are therefore unsuitable for research of this kind. Research into the safety and reliability of blastomere biopsy can generally be conducted using surplus embryos; however, this is not possible if the research is concerned with the safety of polar body biopsy. In order to be able to say anything on this subject, it will be necessary to fertilize the oocytes used for diagnosis and therefore to create embryos for research. The same applies to research concerned with the safety of techniques such as IVM and the cryopreservation of oocytes.

Furthermore, the on average lower quality of surplus embryos is an obstacle to some types of research. For particular research proposals, distortions as a result of the possibly non-representative nature of the IVF population can be avoided by using surplus embryos taken from women with blocked Fallopian tubes.

The research concerned with the combined development of IVM and the cryopreservation of oocytes may result in IVF treatment in the future leading to far fewer surplus embryos than is the case at present, with the result that hardly any surplus embryos will be available for scientific research. The Committee believes it would be justifiable to create embryos for research that as such could be conducted using surplus embryos, but for which no or insufficient surplus embryos are available.

4.4.3 Interests of oocyte-donor candidates

Using surplus embryos for research obviously also requires the informed consent of the persons providing the gametes (usually the man and woman for whom the embryo is created). However, in view of the risks associated with the necessary hormone stimulation and follicle puncture, it is questionable whether it is acceptable to request healthy women to donate mature oocytes for research.

Referring to the analogy of recruiting healthy trial subjects for scientific research that involves certain health risks to the subjects themselves, some members of the Committee believe that making such a request need not be considered unacceptable, provided the criteria of the Medical Research Involving Human Subjects Act are satisfied (EK98). Other members believe that requesting healthy women to donate oocytes for research is completely unacceptable. It is unlikely, though, that many women will be willing to undergo hormone stimulation in order to donate oocytes for scientific research.

The question is therefore whether mature oocytes for research can be obtained in any other way. Three alternatives are available, the first of which also presents a moral dilemma. It would be possible to recruit donors from IVF patients, who must anyway undergo hormone stimulation and follicle puncture for their own treatment. Although these women run no additional health risk if they decide to donate oocytes, they may well reduce their chance of having a child in doing so. The woman may feel unable to refuse a request from her doctor to donate a few oocytes, especially if they were to be used for research into improving artificial reproduction (to assist sister sufferers!).

The Committee believes approaching women in an IVF programme may be acceptable in exceptional cases, namely when a large number of oocytes is obtained *and* a high fertilization percentage is achieved in a prior attempt. No pressure may be placed on the woman to cooperate. The Committee stresses that it is not acceptable to exposé a woman to a heavier stimulation regime than is considered necessary for her treatment, simply in order to obtain a larger number of oocytes with a view to using some of them for research.

Finally, there are two other possibilities that present no moral problems and which are therefore preferable, even if they only offer limited consolation for different reasons. Firstly, women in an IVF programme could be asked permission to use oocytes that turned out not to have been fertilized. Although a second attempt at fertilization could be undertaken, this is not generally considered responsible within the context of treatment. This objection would not apply to a second attempt (using IVF or ICSI) undertaken to obtain research embryos. However, the oocytes may be of a lower quality. Whether or not this is a drawback depends on the nature of the research for which the research embryos are created.

The other possibility would be to ask women who have to undergo a gynaecological operation (e.g. sterilization) for permission to harvest any mature oocyte. Because this involves an unstimulated cycle, most of the aforementioned risks for the donor would be removed. Only a very limited yield can be expected from this option because no more than one mature oocyte can be obtained from each donor.

In summary, the Committee believes that obtaining mature oocytes with a view to creating embryos for research need not be seen as completely impossible, taking into account the interests of the oocyte-donor candidates.

The reference to *mature* oocytes was intentional in the above. After all, in the case of research requiring *immature oocytes,* the last possibility referred to (requesting women who have to undergo a gynaecological operation) is a suitable and unproblematic way of acquiring sufficient research material. Major research proposals that require the use of immature oocytes include *in vitro* maturation, and cryopreservation of immature oocytes.

As mentioned, the research concerned with the combined development of these techniques may result in fewer surplus embryos being available for scientific research. On the other hand, the development of these techniques may bring an end to the scarcity of oocytes for the creation of research embryos, as the latter would no longer require the use of mature oocytes.

4.4.4 *The fourteen-day limit*

The Committee notes that international consensus appears to be growing in favour of the prohibition of keeping human embryos *in vitro* for longer than fourteen days (development period) (GR86, RCN93, War84). It points out the pragmatic nature of this consensus, as there are differences of opinion about the plausibility of the underlying arguments (especially among ethicists), and that some authors call for a later limit (such as a development period of approximately six weeks) (see 5.3.4). As current knowledge does not permit the culture of human embryos for longer than approximately seven days, without them losing their internal structure, the Committee believes it can refrain from pronouncing upon this, especially because the research this advisory report was intended to consider (embryo research in the context of PGD and artificial reproduction) can be conducted without keeping embryos *in vitro* for more than a few days.

4.4.5 *No transfer of embryos used for research*

At the beginning of section 4.5, the Committee stipulated that in embryo research due consideration must be given to the interests of all those concerned. This entails not only considering the persons who have rights in respect of any surplus embryos they donate for research, or (in the case of research using specially cultured embryos) the potential gamete donors, but also the child that might develop from the embryo, if it were to be transferred. Besides the fact that the embryos or gametes have not been donated for this, transfer is unacceptable because of the risk of the research having

harmful consequences for the child. Considering the interests of all those concerned (and in accordance with the criterion that the value of human embryos must be respected) it is necessary to allow any embryos remaining to perish directly after the research has been completed, also in order to prevent their misuse.

4.4.6 Information and consent

In all cases, using surplus embryos for scientific research requires the written consent of those who have rights in respect of those embryos, generally the man and woman for whom they are created. However, in the case of donor IVF, the discretion of sperm or oocyte donors cannot be ignored with regard to embryos created from their gametes. Until new legislation determines otherwise, the point of departure should be that consent must be obtained from those whose gametes are used to create the embryos. It is best to obtain the consent of sperm or oocyte donors in advance (when they donate the gametes), although this does not affect the fact that donors can go back on what was agreed in the donor agreement. See the Committee's previous advisory report (GR97a) for a more extensive discussion of the extent of the donor's discretionary rights.

The woman and man must be provided with sufficient information to enable them to make an informed decision about the possible donation of embryos for research, also bearing in mind the possible alternatives (storage for their own use, donation to another subfertile couple who would like to have children, or allowing the embryos to perish). The information required for this should be provided before the IVF treatment commences. They should be thoroughly informed about the nature and purpose of the research for which the donation is being requested. There must be a possibility of only donating embryos for particular research aims, or only embryos without any normal chance of development. It must be explained to the woman and man that they are entitled to go back on their decision, provided the research has not commenced, but that embryos already being used in research no longer qualify for transfer. The woman and man must not be subjected to any form of pressure. This aspect is particularly important if a treatment relationship exists at the same time. Nor may any payment be made for donated embryos. Embryo donation is not possible if the woman or man are unable to agree on the destiny of their embryos. In that case, the only remaining option is to let the embryos perish straight away.

The specific consent of the sperm and oocyte donor is required in the case of research that does not use surplus embryos but embryos that have been created within the scope of research. The aforementioned criteria for the provision of information also apply in such cases, on the understanding that the information must be provided before the gametes are donated. The criteria of the Medical Research Involving

Subjects Act (EK98) apply to oocyte donation requiring an invasive procedure (i.e. all cases except those in which oocytes are donated in an IVF programme; see 4.5.3).

Finally, the Committee points out that there must be a discussion with the donors of embryos or gametes beforehand to determine whether they wish to be informed of any data relevant to their own health that may become available during the research.

4.4.7 *Central review*

As is clear from the aforementioned ministerial memorandum containing an outline of a new bill (TK95a), the intention is to stipulate by law that all research using human embryos must be presented for review to the Central Committee to be set up in accordance with the provisions of the Medical Research Involving Human Subjects Act. The Central Committee must ensure that the proposed research satisfies all the statutory criteria. This means that assessing the relative weight of the health interests involved in the research is also left to this committee.

The Committee supports the decision to have research reviewed centrally in this field, which is in so many respects a sensitive one (see also GR97b).

4.5 Conclusion

The Committee believes that both the further development of PGD and the improvement of IVF treatment (which may lead to reduced risks for the woman, fewer surplus embryos, and an improvement in the balance between the positive and negative effects of treatment, owing to a greater likelihood of success), is of such major importance for human health, that research using human embryos may be acceptable with these aims in mind. This is not to say that any specific research in this field is acceptable. That also depends on whether the knowledge required to attain these objectives can be obtained in any other way (for example through research using animals). Where the safety of new methods and techniques is concerned, the research must be expected to provide a better-founded answer as to whether it would be responsible to make the step to clinical practice (see 3.5).

In certain cases, depending on the nature of any uncertainty remaining after the completion of animal experiments and the severity of the possible consequences, the Committee believes it would be unacceptable to directly introduce a new method or technique into clinical practice before carrying out research using human embryos, or waiting for the results of such research. Whether this involves research using surplus embryos or research that requires the creation of human embryos is not a deciding factor here (see 4.4.2).

If the outcome of the discussion of the acceptability of embryo research is that creating embryos for research purposes is to be prohibited in the Netherlands, the Committee believes this should not lead to direct testing in clinical research of certain techniques (such as IVM or oocyte cryopreservation) about the safety of which embryo research could provide important information. Under no circumstances whatsoever may women and children be turned into trial subjects for the sake of protecting embryos*.

If any such prohibition is introduced, it would be possible to await the results of preclinical embryo research conducted abroad into the safety of given new techniques. The United Kingdom in particular is one country where the creation of embryos for research is permitted under certain conditions. Albeit with some delay, IVF practice in the Netherlands could then also benefit from any significant improvements resulting from techniques such as IVM or oocyte cryopreservation becoming available. However, it would be folly to believe that this provides an escape from the dilemma outlined here. Those who set their sights on obtaining results abroad from research that is prohibited here deprive themselves of any arguments for credibly supporting that prohibition (Bea96).

* McLaren compared the transfer of embryos that have been exposed to experimental techniques with "making the first test of a new aircraft-guidance system on a crowded Boeing 747" (McL89).

Evaluation

Introduction

With this advisory report the Committee concludes a series of three on artificial reproduction. The earlier reports were on ICSI (July 1996; GR96) and on Standard IVF (February 1997; GR97a). The series deals with the level of knowledge on artificial-reproduction technology as well as the associated social issues.

These advisory reports were commissioned in connection with the intention of the Minister of Health, Welfare and Sport to update the 1989 IVF Planning Decree. Almost ten years later, in 1998, the new Planning Decree is a fact. The Act on which these regulations are based is also new, i.e. the Special Medical Treatments Act, which came into force on 14 November 1997 (Act of 24 October 1997, Stb 1997, 515), and replaced the section 18 regulations of the Hospitals Act.

The importance of government involvement in IVF and the associated techniques is not a point for discussion. In the parliamentary handling of the Special Medical Treatments Act, IVF was referred to on various occasions as an example of a medical procedure that calls for government involvement. Quality assurance and regulation of demand play a role in this, but in particular, also the ethical and legal issues that surround IVF and the techniques associated with it.

In its deliberations, the Committee encountered an issue of a social nature for which it requests specific attention here, in view of future developments in artificial-reproduction technology: to what degree have IVF and related techniques been introduced in the Netherlands in the recent past along the lines of the principle of

'evidence-based medicine' and does the legislation on this subject provide the necessary footing? The question arose because the IVF Planning Decree is the Committee's focal point and regulations of this type are pre-eminently intended as a means of providing guidance in the control of given medical procedures, after their introduction into the health care system. Although the issue is not specifically concerned with artificial-reproduction technology, it is highly significant in this field, because developments are taking place at such a pace and are so far-reaching; it appears as if all the limits set by nature can be exceeded. The Committee realizes that the discussion about the introduction of 'special' medical technology is complex. It does not claim to provide a definitive analysis and vision here. However, it does believe it can provide some key points for the topic's further discussion. To this end, it first outlines the reference model for evaluating 'special' medical technology, with a view to it being introduced judiciously and it provides an insight into the regulatory frameworks. The Committee then provides a brief analysis on the basis of its advisory reports of how reproduction technology has been introduced in the Netherlands and the role regulation has played in this. Finally, the Committee formulates a number of conclusions.

Reference model

When assessing medical technology with a view to its judicious introduction, it is important to establish that it is 'ripe' for use. A number of phases can be distinguished (Bux97, GR88, STG87):

1 The development phase. Fundamental scientific research produces results that may form the starting point for a new medical procedure. This stage is mainly concerned with laboratory research (including research using human gametes and embryos) and animal-based research into the working mechanism, toxicology, etc.
2 The phase in which human subjects are used on a trial scale. It has to be determined in this phase whether the method is effective and safe, ideally on the basis of sound research (RCT). The research will use trial subjects on a limited scale.
3 The phase concerned with the possible incorporation of the new technology into health care. In this phase, the research (ideally RCTs) is concerned with determining the most appropriate indication for referral, the technology's effectiveness (efficacy and cost-effectiveness), and the required scale, distribution and organization.
4 The phase concerned with more wide-spread distribution (diffusion). Various types of evaluation studies are conceivable, such as a study of the possibilities for countering any over- or under-utilization detected in phase 3, or an assessment of

inter-doctor variations (or differences between organizations) in the quality of care provided.

These phases are not always sharply distinguished in practice. Phases one and two are frequently not applied to many types of health care satisfactorily, if at all. The transition to phase three often occurs silently, as is the case with the transition to phase four. Ideally, research should be conducted in all these stages and the next phase should only follow, if the research results of the preceding phase give cause. The judicious and controlled introduction of 'special' medical technology is a complex affair involving many players: the profession, research financiers (including the Netherlands Organization for Scientific Research (NWO), the Investigative Medicine Committee, the Netherlands Foundation for Health Care Research (ZON), institutional review boards, the Health Council, the Health Research Council (RGO), and the government (TK95b).

The regulatory frameworks

The regulatory frameworks are intended to correspond with the ideal picture. The Committee has limited itself here to naming two Acts that it considers important in the introduction of'special' medical technology. These are the forthcoming Medical Research Involving Human Subjects Act and the Special Medical Treatments Act. The former will apply to new techniques that are still at the research-stage (EK98). Research has to demonstrate whether the technique is safe, efficacious and reliable. The Act prescribes an institutional review of the application of techniques within the scope of a protocolled research setting. To this end, the Act provides a recognition system for institutional review boards. The Act also establishes a Central Committee which will, in the fist instance, assess the scientific soundness and ethical acceptability of research for which there is a scarcity of expertise in the Netherlands.

The Special Medical Treatments Act applies to medical procedures in the broader sense of the word (Stb97). This includes both existing and new techniques or methods that are still being developed. The Act provides the Minister of Health, Welfare and Sport with the possibility of subjecting procedures to a licensing system, possibly temporarily. This instrument is comparable with the old system of section 18 of the Hospitals Act. The minister can impose a statutory prohibition on a procedure by means of an Order in Council. Moreover, the Act gives the minister powers in the field of investigative medicine.

Introduction of artificial reproduction techniques in the Netherlands

In delineating the level of knowledge concerning major artificial-reproduction techniques, the Committee has obtained an insight into the way in which these techniques are introduced in the Netherlands and has formed an opinion about the issue of whether they are introduced judiciously.

The Committee finds that IVF has not been introduced entirely in accordance with the reference model (GR97a). This judgement is in line with the 1991 Annual Report on Health Care, which was largely devoted to developments in the field of reproduction (GR92). The main problem was with phase 3: too few randomized trials have been conducted to determine the effectiveness of IVF. The method has rapidly been deployed in clinical practice as an accepted form of health care and, without any justification based on research data, the scope for referrals has quickly widened. Another problem is the lack of follow-up data about children born after IVF. A cost-effectiveness study was carried out as early as 1989. This made a considerable contribution to limiting the number of centres using IVF. The Committee has not concerned itself with phases l and 2 of Standard IVF: this was not part of its remit, since IVF has been considered as an accepted form of health care in the Netherlands since 1989, and a Planning Decree, partly based on a 1986 Health Council advisory report (GR86) was in force.

The introduction of the technique of embryo cryopreservation has also only been partly in line with the reference model (GR97a). The main problem was with phase 2: too little research has been conducted into the safety of cryopreservation. Nor have there been many randomized studies into the technique's effectiveness, phase 3.

With regard to ICSI, MESA and TESE, the Committee has already established that the three techniques are at different stages of introduction in the Netherlands (GR96). Only 'standard' ICSI (using ejaculated sperm) has been deployed to a certain extent and can be considered to be currently in phase 3. In the Committee's view, the animal-based experimental research that preceded the clinical research was extremely limited, particularly the research concerned with the safety of the technique for offspring. Technical problems stood in the way of that research. The step to clinical research (phase 2) was taken quickly, partly for this reason*. ICSI was also rapidly introduced in the Netherlands as a Standard technique (phase 3).

* In connection with this, the Committee refers to recommendations issued by the Provisional Central Ethical Review Board (KEMO) in April 1994 to the IVF centres, following a concrete research protocol for ICSI. On the basis of the level of knowledge at the time, KEMO was largely in agreement with the clinical research proposal. KEMO stressed the uncertainties about safety with regard to offspring.

The Committee considered it responsible to view ICSI as an accepted form of health care because research data on children born after ICSI gave no cause for concern. However, it took the position that the use of ICSI must be subject to strict criteria (especially with regard to referrals) and that follow-up examinations of the children are necessary.

In its advisory report of 1996 the Committee took a different standpoint on MESA and TESE. These techniques were hardly used at all in the Netherlands. The Committee called for further animal-based experimental research into their safety with regard to offspring. The profession also had reservations about MESA and TESE in clinical practice: it introduced a moratorium.

In this third advisory report, the Committee discusses the level of knowledge concerning a number of new techniques that are related to IVF in various ways. It first discussed PGD and the method's three variants. The Committee has found that all the variants are still at the research-stage. In the Netherlands, patiënt studies are only being conducted for blastomere biopsy. The research protocol on which this is based has been approved by the Provisional Central Ethical Review Board (KEMO) (KEM91, KEM94) and is currently in phase 2: patiënt studies are being conducted to obtain an insight into the technique's safety, effectiveness and reliability. The Committee believes blastomere biopsy has been introduced judiciously in the Netherlands thus far.

The Committee believes further animal-based experiments and preclinical research using embryos (phase 1) must be carried out for the major new techniques referred to in this advisory report, to wit, IVM and oocyte cryopreservation, before they can be responsibly deployed in clinical research.

As is clear from the above, the Committee believes the question of whether artificial-reproduction techniques have been introduced judiciously in the Netherlands cannot be answered with a simple 'yes' or 'no'. The picture varies. A degree of perspective is also necessary because in practice it is never possible to satisfy all the criteria of a reference model. It is also pointed out that practice in the Netherlands is unavoidably influenced by surrounding countries. Artificial-reproduction techniques also involve some special aspects in view of which it is not always easy to follow all the phases of the reference model. It has become clear that scientific research using human embryos (preclinical research) will be necessary within the context of phases l and 2. This is a controversial issue. Artificial reproduction also often involves an urgent request for help from patients who believe everything should be done to fulfil their desire to have children, and the medical profession wants to help them succeed. The necessary cautious research steps are therefore sometimes taken too quickly, or missed out altogether.

The set of statutory Instruments

The Committee believes the IVF Planning Decree based on the Hospitals Act has kept a grip on any expansion of IVF treatment (phase 4). Concerning the medico-ethical assessment of ICSI and PGD, an advisory role has been taken on by KEMO. The aforementioned new legislation will provide the government with more instruments for promoting the judicious introduction of technology (EK98, Stb97). The Medical Research Involving Human Subjects Act provides criteria for assessing concrete research proposals (as the future legislation on the use of human gametes and embryos is also expected to do), and it is reasonable for the Central Committee to be appointed as the evaluating body for research proposals concerned with artificial reproduction. The Special Medical Treatments Act enables the Minister to issue a prohibition on the use of a new technique in clinical practice. The Committee assumes that this offers a possibility for preventing the step to incorporation in Standard health care being taken too soon.

Conclusions and recommendations

The introduction of 'special' medical technology is complex. Given a number of special aspects, this is all the more so for new artificial-reproduction technology. In spite of warnings in advisory reports from the Health Council that have been taken into account in various sections of regulations, the introduction of artificial-reproduction techniques in the Netherlands has not been entirely satisfactory. The Committee's most important point of criticism pertains to the omission of steps in the requisite research: animal-based experimental research, preclinical research using embryos, randomized trials with patiënt groups, and follow-up research among the children. In the Committee's view, the development and possible introduction of future techniques should be more along the lines of the reference model than is the case at present. It believes the aforementioned new legislation may provide a useful instrument for achieving this. However, the Committee points out that research is impossible without the finances to resource it. The Committee believes the financial implications of judicious phasing should also be paid particular attention when setting priorities for research. Prioritizing is a joint responsibility of government, the profession and research financiers. The Committee's view is that where assisted reproduction is considered socially important, the judicious development of the necessary knowledge and techniques should also be facilitated. The Committee notes in agreement that the current policy stresses the principle of 'evidence-based medicine' with regard to the introduction of new technology in health care. This principle should also be

the starting point in the field of artificial reproduction, all the more so because in this field it is always also the health and other interests of the offspring that are at stake.

Rijswijk, 19 March 1998,
on behalf of the Committee

(signed)

JGM Aartsen, scientific secretary	W J Dondorp, scientific secretary	professor J W Wladimiroff, chairman

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Annexes

Annex **A**

The request for an advisory report

On 24 February 1994, the then State Secretary for Welfare, Health and Cultural Affairs wrote as follows to the Health Council (letter ZZT/TOPAZ/ 941055):

With a view to updating my policy on artificial reproduction, in terms of the application of section 18 of the Hospitals Act, I hereby request you to inform me about the present level of medical science in this field. In any case, this concerns the In Vitro Fertilization Planning Decree.

I have already received advisory reports from you on artificial reproduction. The interim advisory report that was mainly concerned with the medico-technological aspects of in vitro fertilization was published on 10 October 1984 and was followed on 16 October 1986 by the advisory report on artificial reproduction, which was mainly concerned with in vitro fertilization, artificial insemination using donor sperm, and surrogacy. Your 1991 annual report on health care also focused on reproduction.

One of the measures taken on the basis of your advisory reports was to make the use of in vitro fertilization subject to section 18 of the Hospitals Act. This at present includes the following specific regulations:

1 The decree concerning the Hospitals Act's regulations on the designation of institutions, section 1, subsection E.5, in which "laboratories where human embryos are produced and stored" are now designated as hospitals within the scope of the said Act. This makes the compulsory licensing for IVF applicable to all laboratories covered by this description, including private clinics.

2 The decree concerning the Hospitals Act's regulations on special procedures and equipment. These regulations prohibit unlicensed hospital facilities from being used for 'in vitro fertilization

techniques, insofar as this involves the production of human embryos outside the body'. With this decree, in vitro fertilization is made subject to the provisions of section 18 of the Hospitals Act for an unspecified period.

3 The In Vitro Fertilization Planning Decree, which regulates the demand for laboratories for in vitro fertilization and the way that demand can be fulfilled. See Netherlands Government Gazette 146, 1989 and, for the amendment decree of 10 November 1989, see Netherlands Government Gazette 102, 1990. During 1990 and 1991, these regulations led to IVF licences being issued to 12 centres, the 8 university hospitals and 4 general hospitals.

The designated IVF centres work on the basis of a treatment protocol, which is submitted to the Medical Inspectorate of Health. Some centres operate joint ventures with transfer hospitals, which have to conform with the relevant treatment protocol. The joint ventures are governed by written joint venture contracts, which are also sent to the Medical Inspectorate of Health. Satellite clinics have also existed for some time now. These operate on the basis of joint ventures that are not covered by the provisions of the Planning Decree.

There has been a sharp increase in the number of treatments over the past few years. In 1991, 7600 treatments were provided, whereas the figure for 1993 was more than 8500. The Planning Decree is based on an estimated maximum of 4500 treatments per year.

Besides the sharp increase in the number of treatments and partly in connection with this, developments have also taken place in the indications for referrals and in IVF technology, or the combination of the two. Current uses include IVF if the man is infertile, if the woman is well over 40, and also in combination with pre-implantation diagnostics. There are also variants of artificial fertilization, which use techniques that are comparable with in vitro fertilization.

On the basis of these and other developments, I believe the IVF Planning Decree needs to be updated.

I would like you to produce an advisory report that at least covers the aspects of demand and quality that concern your council, as currently laid down in the IVF Planning Decree. I also request you to consider the type of registration required in terms of content, and any possible research within the scope of either the 5% rule or investigative medicine, insofar as this is relevant for the section-18 regulations. If necessary, updating the regulations may also be considered to include broadening the scope of section 18 of the Hospitals Act to include techniques that are comparable with in vitro fertilization. However, I would err on the side of reticence in subjecting any new techniques to the provisions of section 18, in view of the stringent central regulations the section involves. In my view, section 18 need not be applied wherever self-regulation is working properly.

I would also like to point out the following with regard to the link with future legislation on fertilization techniques. In anticipation of medical ethics legislation, some medico-ethical aspects of in vitro

fertilization are covered by the IVF Planning Decree. I also intend to do likewise in the new regulations, while awaiting the completion of the said legislation, although any such legislation will not necessarily result in the withdrawal of the section-18 regulations. Section 18 regulates different aspects from those of the proposed legislation. After all, the section-18 regulations are concerned with bringing about a concentration, in connection with quality and/or costs. I do not exclude the possibility of the medico-ethical legislation and the section 18-regulations co-existing.

The following questions are based on the above considerations:

- From the point of view of the level of medical science, are there any reasons to broaden the scope of section 18 to include other methods of artificial reproduction and/or the associated research and treatment methods?
- Could you provide an estimate backed up by figures to indicate the demand for IVF and its uses up to at least 1999?
- From the point of view of the level of medical science, are the conditions laid down by the present regulations adequate, with regard to, for example, the indications for referral, quality aspects, and the success percentage? See in particular section IIB of the appendix to the Planning Decree.
- Are there any reasons for changing the present medical registration system in order to enable adequate monitoring?
- Do you believe any policy research or investigative medical research is necessary for particular aspects?
- Could you indicate any possible new ethical or social issues that may relate to the developments being considered?

For the purposes of the advisory report, I request you to appoint Ms CM Trentelman as the advisor for the Ministry.

I would like to receive your advisory report no later than during March 1995.

The State Secretary for Welfare, Health and Cultural Affairs,

(signed) Hans J Simons

Annex **B**

The Committee

- JW Wladimiroff, *chairman*
  professor of obstetrics and gynaecology; Dijkzigt University Hospital, Rotterdam
- TAM Te Braake
  health lawyer; University of Amsterdam
- AML Broekhuijsen-Molenaar
  lawyer; Leiden University
- S Buitendijk
  epidemiologist; TNO Prevention and Health, Leiden
- JDF Habbema
  professor of medical decision theory; Erasmus University Rotterdam
- CJCM Hamilton
  gynaecologist; Groot Ziekengasthuis, 's-Hertogenbosch
- RM Den Hartog-van ter Tholen, *advisor*
  Ministry of Health, Welfare and Sport
- JP Holm,
  professor of obstetrics and gynaecology; Groningen University
- CAM Jansen
  gynaecologist; Diaconessenhuis, Voorburg
- MHEC Pieters
  embryologist; Rotterdam University Hospital
- ER teVelde
  professor of reproductive medicine; Utrecht University Hospital

- J Vermeiden
  embryologist, University Hospital of the Free University, Amsterdam
- GMWR de Wert
  medical ethicist; Institute for Bioethics, Maastricht; Erasmus University, Rotterdam
  JGM Aartsen, *scientific secretary*
  Health Council, Rijswijk
- WJ Dondorp, *scientific secretary*
  Health Council, Rijswijk

The Committee sought advice from:
- JPM Geraedts
  professor of genetics and cell biology; Maastricht University
- R Gosden
  professor of reproductive biology; University of Leeds (UK).

Secretarial and administrative assistance: M Weurman-Ubels.

Annex **C**

Medical terminology used in this advisory report

Allele

A chromosomal pair contains two genes for each genetic characteristic. The two genes, one from each parent, may differ in their form and are known as alleles. When the alleles are identical the individual is known as a homozygote (for the characteristic concerned), and when different as a heterozygote.

Aneuploidy

A condition in which the number of chromosomes in the cells differs from the normal number. In most cases the condition occurs because the two halves of a chromosomal pair have not divided correctly during reduction division*. This results in a chromosome that ought to occur in somatic cells in pairs occurring instead as a single chromosome (monosomy) or in triplicate (trisomy). A familiar example of the latter is trisomy 21: Down's syndrome. The condition in which all chromosomes occur in triplicate is known as triploidy. Triploidy usually occurs because the oocyte is not fertilized by one but by two sperm cells.

Autosomal recessive hereditary condition

A monogenic* condition caused by an abnormal gene on one of the autosomes (= all chromosomes with the exception of the sex chromosomes). The word recessive indicates that the mutation in question

has to occur on both alleles* of the gene in order to be expressed. In dominant hereditary conditions, the abnormality is always expressed if the mutation is present.

Blastocyst

Name given to the embryo at the stage before implantation. A cavity forms (blastocoele) with a surface layer of cells (trophoblast) on the outside, and a cell mass (embryoblast) on the inside. The tissues of the embryo proper develop from the inner cell mass. The cells of the trophoblast contribute to the development of supporting tissues, including the placenta. Human embryos reach the blastocyst stage approximately 5 to 6 days after fertilization.

Blastomere

Name given to the cells that make up the embryo during the cleavage stage. See under embryonic development.

Chromosomal abnormalities

Abnormality in the normal chromosomal pattern in the cells. Numerical abnormalities involve an abnormal number of chromosomes (see under aneuploidy). Structural chromosomal abnormalities are caused because breaks that occur in the chromosomal material fail to repair properly. Various types of abnormalities may arise depending on the number and location of the breaks.

Crossing over

The first reduction division of the gametes produces two daughter cells (including the first polar body* in the case of female gametes) each of which contains half the genetic material of the parent cell. Alleles* may be exchanged between the two halves of the chromosomal pair during the division of the genetic material. This process is called crossing over.

Embryo

The name given to the developing organism until eight weeks from conception, after which it is referred to as a foetus up to the time of birth. During the first fourteen days of an embryo's development the name 'pre-embryo' is sometimes used (but not in this advisory report).

Embryonic development

Until implantation in the uterine wall, the embryo* passes through the following stages of development. The fusion of the gametes produces a zygote. This is followed by the cleavage stage, in which the number of cells (blastomeres) increases rapidly, without the embryo growing in size. When it consists of 12 to 16 cells, it is referred to as a morula (3 to 4 days after fertilization). An *in vivo* embryo is then on the point of reaching the womb. The morula develops into the blastocyst* (5 to 6 days after fertilization). Implantation can follow once the embryo has grown out of the oolemma *(hatching).* In this process, the outer cells (trophoblast) penetrate the mucous membrane of the uterine wall, to assist in the formation of the placenta. Implantation (nidation) begins on approximately the 7th day after fertilization and is only completed around the 14th day. The primitive streak, which indicates the axis of the embryo, forms shortly thereafter in the inner cell mass.

Genomic imprinting

Modification of the DNA that results in the expression of certain genes being determined by the allele* from either the father or from the mother. A disturbance of *genomic imprinting* can result in various developmental defects, some of which only come to light later in life. There is still a lot of scientific controversy about the course and significance of the *genomic imprinting* process.

Sex-linked (recessive) hereditary condition

A monogenic* condition caused by an abnormal gene on the X- (or Y-) chromosome. If the mother is a carrier of a condition linked to the X-chromosome, she has a one in two risk of passing on the mutated gene. Girls who inherit the mutation will not develop the condition but will be carriers; boys with the gene always develop the condition itself.

Cleavage

See under embryonic development.

Monogenic condition

Hereditary condition based on a mutation of a single gene.

Mosaicism

Name given to the situation in which an embryo exhibits groups of cells of a different chromosomal composition.

Nidation

Implantation; see under embryonic development

Polar body

The first polar body is produced as a result of the first meiotic division* as a sister cell of the oocyte (secondary oocyte). It degenerates upon fertilization. The oocyte completes the second reduction division immediately after penetration by the sperm cell, and the *second* polar body is produced.

Recessive hereditary condition

Monogenic* condition that produces disease symptoms in an individual who has received the associated gene mutation from both parents. The mutation must be present on both alleles* of the gene in order to be expressed.

Meiotic division

A special type of cell division which results in the gametes having half of the number of chromosomes that is normal for somatic cells. This occurs in two stages: the first and second meiotic division.

Trophoblast

See under blastocyst.